WORKING PAPER 44

Western Hemlock
A Manual for Tree Improvement Seed Production

2000

BRITISH COLUMBIA
Ministry of Forests Research Program

100-04993/

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Citation
Webber, J. E. 2000. Western hemlock: a manual for tree improvement seed production. Res. Br., B.C. Min. For., Victoria, B.C. Work Pap. 44/2000.

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Copies of this report may be obtained, depending upon supply, from:
Crown Publications
521 Fort Street
Victoria, BC V8W 1E7
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ACKNOWLEDGEMENTS

I wish to acknowledge all those who have made a significant contribution to making this manual possible. First, to Roger Painter for his dedication, field technique, and data management, which have made it possible to base many seed orchard management decisions on sound technical data. Also, to all of the coastal western hemlock seed orchards; in particular: Western Forest Products; Lost Lake Seed Orchards; Western Forest Products (formerly Pacific Forest Products); Saanich Seed Orchards; and TimberWest, Mount Newton Seed Orchards. The author greatly appreciates the kind permission of Drs. Anna Colangeli (Camosun College, Victoria) and John Owens (University of Victoria) as well as the National Research Council of Canada for allowing me to reproduce figures 14, 19, and 28 from the *Canadian Journal of Forest Research* article titled "Postdormancy seed cone development and the pollination mechanism in western hemlock (*Tsuga heterophylla*)" (19:44–53). The services of R.W. Holcomb of Holcomb Communications (Brentwood Bay, B.C.) as compiling editor are appreciated. Finally, I would like to thank Forest Renewal BC for the award (Operational Tree Improvement Program project number 202) and the support of the B.C. Ministry of Forests.

The Forest Genetics Council has laid out objectives for western hemlock seedling requirements up to the year 2007. These objectives include goals for both the number of seedlings and their **genetic worth**. The only current practical options for meeting these objectives are seed production and a limited amount of **bulking** through rooted cuttings. Rooted cuttings technology is more costly and relatively new. Performance of seedlings from cuttings remains largely untested although cuttings/seedlings comparisons are in place. For the near future, seed production will be our prime source of reforestation **propagules**. This manual compiles information from the Ministry of Forests research program as well as other allied programs to make recommendations for seed orchard production of high genetic gain seed lots.

With our current operational seed production capabilities, meeting requirements for numbers of seedlings will be a relatively straightforward task. Meeting goals for genetic worth is more problematic, and will require innovation in two directions:

- developing technologies based on what we have learned, and
- vigorously integrating these technologies into existing and future seed orchards.

Western hemlock lends itself easily to the first of these innovations because it is one our easiest conifer species to manage. The species (1) grafts well, (2) has crowns that are easily pruned for management as well as seed and pollen cone development, (3) is among the easiest species in which to induce flowering, (4) produces pollen that is relatively easy to handle and stores well for at least 3 years, and (5) does not suffer from severe competing pollen clouds (**contamination**) arising from stands surrounding most western hemlock seed orchards in British Columbia.

This manual includes research summaries for cone induction and crown management and actual research results for pollen management techniques that include:

- evaluation of pollen storage methods,
- comparison of germination techniques,
- development of three pollen viability tests,
- the relationships between viability tests and field fertility, and
- supplemental pollination (SP).

Our research has enabled us to formulate technologies that can be used in existing seed orchards and indicates directions for new orchards. However, our most severe limitation to developing technologies for producing seed of the highest genetic worth is determining supplemental pollination efficacy. At this time if we use pollen from quality lots of known **breeding value** we cannot accurately predict how much of the applied pollen will end up as seed.

A major unknown in this process is the effect of competing pollen. We need to complete trials under various pollen loads to develop supplemental pollination techniques that work for western hemlock. When these pollination techniques are considered in conjunction with management techniques already developed, we will be able to design orchard systems that will help us meet our target goals for high genetic worth seed lots. In the meantime, if we vigorously utilize recently developed techniques in existing orchards, significant gains in genetic worth are possible.

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1 INTRODUCTION

This manual is intended to help orchard managers and tree breeders of western hemlock produce adequate quantities of seed with optimal genetic worth. Both published and unpublished information based on field studies in coastal container- or soil-based seed orchards is presented. Most of the material is on research involved with **cone induction** and **pollen management**, and there are also shorter sections on **crown management** and orchard strategies. It is by combining management of crowns, cones, and pollen with orchard strategies that optimum genetic worth can be achieved.

Protocols presented in this manual are not to be accepted uncritically. While it is reasonable to assume that results from a limited number of coastal orchards are applicable in a general way to other orchards in the Pacific Northwest, different site factors (e.g., meteorological and soil conditions) may vary enough between orchards to make direct application of specific protocol inappropriate. This is especially true for dates associated with the **phenology** of pollen or seed cones and to dates for cone induction treatments. Where an orchard manager is concerned that these protocol do not apply to their orchard or conditions, then we recommend that the protocol be used as a reference point to test and develop orchard-specific procedures.

This manual is written with the assumption that the reader has a fundamental knowledge of the reproductive biology of western hemlock. A detailed account of this subject is outside the scope of this manual, but a brief summary of the essential points along with references for further reading is presented in Appendix 1.

Appendix 2 provides technical details of laboratory viability assays and Appendix 3 provides a glossary of technical terms used in this manual. The first time a term appears it is in **bold**.

1.1 Forest Genetics Council of British Columbia

The responsibility of managing the genetics of forest trees lies with the Chief Forester of British Columbia. It is the Forest Genetics Council (FGC), formerly known as the Tree Improvement Council, that is charged with the responsibility of developing an organizational structure for the efficient delivery of the Forest Genetics program. The approach of the FGC is stated within the strategic business plan with specific objectives for the next decade. This plan is published as Forest Genetics Council Strategic/Business Plan 1998–2007, and is hereafter referred to as the FGC Plan.

The overview in this plan is that "forest gene resource management is the conservation, controlled use, and enhancement of the genetic diversity of B.C. tree species which have, or may acquire, commercial value. Included are stewardship functions of gene conservation, monitoring, and setting **seed transfer guidelines**, as well as genetic improvement to obtain higher value from the resource."

1.2 Objectives

The objectives for western hemlock orchards are the same as for most conifer seed orchards: to supply abundant quantities of seed with the optimal genetic worth. Two objectives of the FGC Plan that have direct effects on western hemlock seed orchard production are:

- doubling the average gain of genetically improved seed from 6 to 12% by the year 2007, and

- increasing the proportion of genetically improved seed used for sowing to 75%.

Within the FGC Plan are a "Genetic Improvement Strategy Timeline," a "Gene Resource Management Plan," and an "Activities and Costs" schedule for low and high elevation western hemlock. These plans specify the timeline for developing the high gain parents and briefly mention the principal source of propagules. The FGC Plan does not provide details about how this elite breeding material can be utilized to increase average gain or increase the percentage of genetically improved seed. It is the purpose of this manual to provide this detail.

2.1 Distribution and Economic Importance

Western hemlock (*Tusga heterophylla* (Raf.) Sarg.) grows along the Pacific Coast from Alaska to northern California with scattered pockets in the interior of British Columbia and the northwestern states. Its light colour and relatively straight grain make it an attractive wood for finishing and pulping. It is an important commercial species for both coastal and interior British Columbia, making up about 40% of the coastal harvest and about 60% of the province's export market. Sowing requests for 1997 were 7.9 million, with the bulk of planting (about 5.5 million) occurring on low elevation, south coast sites.

Because of its prolific regeneration capacity, many western hemlock areas could be allowed to reforest naturally. However, this regeneration capacity combined with the biological diversity displayed by western hemlock also makes it a good candidate for tree improvement.

2.2 Economics of Genetic Gain

The FGC Plan has devised "Business Plan Value Rankings" and ranks the investment opportunity for south coast (south maritime, low elevation) western hemlock fifth among all tree improvement species in British Columbia (FGC Plan 1998). As a program, the incremental value of tree improvement for western hemlock is ranked third behind coastal Douglas-fir and Prince George lodgepole pine. The return on the investment for improving western hemlock is clear. Furthermore, the economic return of using improved seed for reforestation is additionally compensated for by faster green-up and evenly spaced plantations at rotation.

2.3 Tree Improvement Research

In British Columbia, tree improvement of western hemlock began in the early 1970s and has now grown to include about 1400 selected trees (King et al. 1997). This program is part of the larger Western Hemlock Tree Improvement Cooperative (HEMTIC), an organization that includes about 14 government and private forest company agencies throughout the Pacific Northwest (King et al. 1997). The stated objective of this program is to identify elite parents that will be used for seed production of well-adapted **progeny** that can provide optimal **genetic gain** for wood quality and quantity. Breeding programs both in British Columbia and co-operatively throughout the Pacific Northwest (King and Cress 1991) are now fully in place. Results from the HEMTIC program will not be realized for several more years. However, production from earlier established seed orchards has contributed to 41, 48, and 44% of the annual sowing requests in British Columbia for 1996, 1997, and 1998, respectively (SPAR nursery extract files, B.C. Ministry of Forests).

2.4 Genetic Worth

The genetic quality of orchard seed lots is comprised of three components: genetic worth, **adaptation** to the planting zone, and **genetic diversity**. Genetic worth is a quantifiable estimate of average breeding value of contributing orchard parents weighted by clonal gamete contribution and adjusted for contamination and supplemental pollination. For untested seed lots, adaptation of an orchard seed lot is measured by seed transfer guidelines. For parents under progeny tests, adaptation is measured as the mean latitude, longitude, and elevation of the contributing orchard parent **ortet** location. Genetic diversity is a reflection of the number of contributing parents to the seed lot and is measured by effective population size (Ne). The detailed approach for seed lot rating of genetic worth is provided by Woods et al. (1996).

2.5 Orchard Production

In British Columbia, we currently distinguish three seed zones for western hemlock:

1. maritime low elevation (<600 m)
2. maritime high elevation (>600 m)
3. Queen Charlotte Islands low elevation (<600 m).

According to the species plans for western hemlock (FGC Plan 1998) the current level of genetic worth for Maritime low elevation western hemlock is between 5 and 10%, with anticipated levels rising to 15% by 2002 and 20% by 2007. For high elevation hemlock in the Maritime zone, the corresponding values are about 2% currently and are expected to rise to about 5% by 2007. For the Queen Charlotte Islands, only 2% gain is noted for all years up to 2007.

According to the Fifth Progress Report of the Coastal Tree Improvement Council (Reid and Crown 1995), there are 11 western hemlock orchards registered for seed production. However, the more recent species plans for western hemlock (FGC Plan 1998) list eight orchards for the Maritime low seed zone and five for the Maritime high seed zone.

All are currently soil-based orchards. However, at least two orchards were operated as container orchards (orchards 150 and 163, MacMillan Bloedel). These latter two orchards were dismantled because production requirements for both seed quantity and genetic gain were being adequately met in the less costly soil-based orchards. More recently, second-generation orchards have been established at TimberWest (Mount Newton), Canadian Forest Products (Sechelt), and Western Forest Products (formerly Pacific Forest Products) (Saanichton), with the latter designed as a clonal-row orchard. These orchards will be the main source of improved seed when production begins in a few years.

With the current number of orchards, overproduction of lower genetic worth seed lots is likely. As higher breeding value parents are developed, incentives to increase genetic worth using alternate delivery systems (e.g., vegetative bulking and **clonal deployment**) could further reduce our reliance on orchard seed. If seed orchards are to be more than just a vehicle for generating elite controlled-pedigree seed lots, then management principles must be directed towards the optimization of both the quantity and quality of production seed lots.

There are a number of options available to the orchard managers, which we will discuss in the section "Orchard Strategies." For all strategies, orchard managers must stress the genetic value of production crops. Seed lot rating protocols are being used to evaluate the genetic worth of all crops, and their value will be directly related to either maintaining or improving genetic worth. We will not discuss the rating protocol (Woods et al. 1996) further but rather emphasize those aspects of the protocol (e.g., supplemental pollination) that can be manipulated to improve the genetic worth of existing and newly established orchards.

2.6 Improvement and Potential Gain

Western hemlock is known to be both a good cone producer and a prolific seeder, at least for low elevation sites (Fowells 1965; Owens and Molder 1984). Cone crops occur yearly, with good crops occurring every 3 or 4 years (Fowells 1965). Reports of millions of viable seeds per hectare being released from mature western hemlock trees are common. Obviously, reproductive potential in western hemlock is relatively easy to attain.

The objective is to utilize this reproductive potential to achieve genetic gain, and this can be done by adopting a management strategy that includes some form of plantation forestry. Whether the source of improved propagules is vegetative propagation (rooted cuttings) or sexual reproduction (seed orchards) will be determined by the development of appropriate technologies and costs. Certainly western hemlock's potential for flowering and seed production makes seed orchard production a very attractive option.

Our current seed orchard strategies can produce adapted plantation stock with a genetic gain potential approaching the average breeding value of the orchard parents. With the inclusion of intensive seed orchard management and higher breeding value parents, seed yields and their genetic worth will increase.

3.1 Introduction

Seed orchard managers will be challenged to incorporate better breeding value parents into their current orchards while keeping production schedules at target levels. In general, current orchard designs do not lend themselves to these objectives.

Initially, orchard design and size were determined by projected planting requirements and expected yields based on relatively conservative estimates of production. In almost all cases, orchard production began sooner and produced more per **ramet** than estimated. As a consequence, orchards tended to be larger than necessary. Also, as new seed orchard planning zones were developed and potential seed production estimates updated, the size of and number of parents within these orchards became considerably smaller (Hanson 1986). Wind pollination within these smaller orchards could not be relied upon (Woessner and Franklin 1973) and intensive management practices, including pollen management, were required.

These management practices add costs, but with the introduction of incentives (seed lot price, annual cut allotments, and access to adjacent stands) interest in producing high genetic worth seed lots has increased. Now, decisions about orchard systems and their design, as well as orchard management, will play a more important role. Orchard systems involve choices between a field-based or a container-based system and orchard design involves choices about location and spacing of ramets. Orchard management involves decisions about crown management, cone induction, and pollen control. Orchard strategies involve the interplay between orchard design and orchard management.

Under seed orchard conditions, western hemlock can flower early and abundantly and responds well to crown management techniques. Some of these strategies are currently being used for hemlock seed production and others should be considered for use. Orchard design is probably the least appreciated strategy for facilitating orchard production, while orchard management techniques have been fully employed. We will briefly summarize information about orchard systems, their design, and their management. The remaining part of this manual will then focus on orchard management practices.

3.2 Orchard Systems

The two options for seed orchard production systems are container- and soil-based. Ross et al. (1986) and Eastham and Ross (1988) offer a detailed discussion of the potential advantages of the container seed orchard. These include better production (earlier and more abundant flowering with fewer pest problems) over a smaller land area and improved genetic efficiency (isolation from contamination and increased control over parental contribution). Also, new breeding material (seed cone trees and pollen) can be infused rapidly into the production population as it becomes available.

There are risks, however. Container technology has been available for a number of years but orchard managers have been reluctant to use this orchard approach. Potted trees are more sensitive to rapid changes in their environment and are less forgiving of mismanagement or lack of attention. Container seed orchard technology has been used for western hemlock seed production (MacMillan Bloedel orchards 150 and 163) but the added cost of production was not justified by the low breeding value of available parents. This may change as better breeding value parents become available.

Container seed orchards are being used extensively in the United States for western larch and to a limited extent for western hemlock. Container seed orchard technology for seed production of western hemlock was the most successful for all species tested and still remains a viable option for limited seed production needs in British Columbia. However, before considering this option for seed production, managers should be well versed in the basic horticultural technique of pot culture, cone induction procedures, and seed cone maturation techniques.

3.3 Orchard Designs

Soil-based orchards of traditional design will continue to be our principal source of seed for the foreseeable future and they likely will continue to be predominantly traditional designs. Traditional orchards were designed to maximize **out-crossing** with the assumption that management of crowns, cones, and pollen would be minimal. Ramets of clones were placed randomly throughout the orchard, and wind pollination was allowed to proceed. Initial spacing varied from 2 to 16 m depending on the long term objectives of orchard production. Each unit contained 50 or more clones and ramets were placed in several randomized designs (Giertych 1975). The initial strategy for managing randomized block designs was to minimize inbreeding but it placed no emphasis on controlling out-cross pollen. However, because the timing and amount of seed and pollen production differs from clone to clone, random-design orchards tend to partition into discrete populations due to different dates (phenology) of receptivity (e.g., early, mid, and late).

As the number of orchard parents become fewer, this partitioning and the lack of control over out-crossing makes sustained production of high genetic worth seed lots more problematic and costly. Our seed production priorities are now shifting towards capturing gain. Traditional orchard designs are difficult to improve and do not lend themselves to intensive orchard management. Orchard ramets are being top pruned to give better access to the crop but it is the clonal row orchard design that offers more flexibility in controlling the parental structure (pollen) of production seed lots.

Clonal-row orchards are simply orchards designed with all ramets in a row to facilitate intensive orchard management practices. Normally, they are more closely spaced (2–4 m) since crown pruning controls crown spread. They are more like a breeding orchard in design but are managed for production as well. At least one clonal-row orchard for western hemlock has been established by El-Kassaby (1997) at the Western Forest Products (formerly Pacific Forest Products) Saanich Seed Orchard site. One of the major concerns with clonal-row orchards is that their design promotes **self-fertilization**. El-Kassaby (1997) compared mating patterns and seed yields to a traditional random-designed orchard and found that both orchard designs produced similar yields but that the out-crossing rates were lower for the clonal-row orchard (0.899 versus 0.997), suggesting that selfing was higher. Supplemental pollination was not considered in this test but El-Kassaby (1997) recommends it as a necessary practice to reduce selfing.

3.4 Current Trends

There has been considerable interest in the developing vegetative bulking-up procedures of specific crosses with exceptionally good breeding values. Weyerhaeuser's Douglas-fir program has been bulking-up about 1 million elite propagules yearly (Ritchie 1993). However, the added cost of this procedure limits its application to only the highest genetic worth seed lots.

In British Columbia, we are also developing bulking-up techniques for most species, including western hemlock. We are still at the testing stage, and full-scale deployment will not be considered until costs and seedling performance are fully evaluated. However, it is likely that vegetative amplification will become an integral part of western hemlock propagule production and we should begin to prepare our orchard production strategies to include **controlled-pedigree orchards**. Clonal-row and crown-pruned orchards should also be seriously considered.

3.5 Orchard Management

The major components of orchard management are crown management, bloom delay, cone induction, and pollen management (supplemental pollination). These four components are defined briefly in the following two paragraphs and three of them are discussed in detail in Section 4.

Crown management involves pruning crowns back to manageable size while maintaining adequate seed and pollen cone development. Western hemlock is very amenable to crown management. With other conifer species (in particular coastal Douglas-fir) it is necessary to delay seed cone development in order to reduce pollen contamination. This is not a concern for most western hemlock orchard sites, so slowing seed cone development is not discussed in this manual. Cone induction is possible with a number of treatments; many include using mixtures of the plant hormone **gibberellins** (specifically a mixture of gibberellin A4 and A7 or GA4/7). Such treatments can cut many years off the time required for naturally induced seed cone crops.

The most versatile tool for orchard management of western hemlock is pollen management. This can involve either **supplemental pollination** (SP) in which relatively large amounts of pollen are used or **controlled pollination** (CP), which utilizes smaller amounts of pollen and normally involves some form of seed cone protection (e.g., bagging).

The increasing emphasis on maximizing yields with the highest genetic worth has turned our attention towards novel orchard designs and intensive seed orchard management. Of the various management techniques we have mentioned, crown management, flower induction, and supplemental pollination are the most useful. By far the most versatile of these techniques is supplemental pollination. The path to successful supplemental pollination is pollen management, and most of the following discussion is given to this topic. Experimental results are given in order to provide managers with the background research upon which we made the recommendations for management decisions. Technical details for testing pollen viability are given in Appendix 2.

4.1 Crown Management

Normally, grafted stock does not flower profusely for 4–6 years after establishment. By this time the ramets are 3–4 m tall. Trees this large have the advantage of producing large seed crops, but the crop is difficult to manage. It is now common practice to at least top prune large trees or in some cases to prune for specific types of branches with a higher potential to produce seed and pollen cones. This pruning is less detrimental for western hemlock than for most conifer species because western hemlock typically has sparse crowns with few potential cone sites. When used in conjunction with routine cone induction, the cone-forming part of the crown can be easily maintained within 2–3 m of the ground.

Studies with other conifer species have shown that removing 40–60% of the live crown can effectively control height without significantly losing cone production (Matheson and Willcocks 1976; Nienstaedt 1981; Masters 1982; Philipson 1985). Ross (1989) has shown this to be true with western hemlock as well. At age 5–6 years, rooted cuttings of a clonal western hemlock orchard were either severely (50% of the live crown removed) or moderately (25% of the live crown removed) pruned. Crop production over the next 6 years showed no effect on either pollen or seed cone production.

Clearly, crown forming is a practical technique for intensive management. Removing the strongly vegetative terminal and lateral shoots encourages more shoot development in the upper crown where seed cones are borne on moderate to vigorous shoots (Owens and Molder 1974). Repeated pruning also encourages the production of less vigorous shoots in the mid and lower crown, which become high potential pollen producing shoots (Ross 1989). Furthermore, Ross (1989) did not find any effect of pruning on induction response. Both seed and pollen cone buds were significantly enhanced the years following spray application of gibberellins.

Similar results have been shown for potted western hemlock stock as well. In fact, for younger orchards, container stock can out produce their soil-based counterparts (Bower et al. 1986; Eastham and Ross 1989), especially if production is based on orchard area and not on individual trees. One of the important reasons increased production can occur is the ability of maintaining crown form and greater control over annual flower induction.

For container stock (Bower et al. 1986), crown forming began the year after grafting and simply involved removing about 50% of the vigorous terminal and lateral shoot growth (February/March) to encourage further lateral and secondary shoot growth. It is far less severe to prune as part of

graft establishment than to remove approximately 50% of the live crown in older material. In the Ross (1989) study, 25 or 50% of the live crown was removed in February, depending on the severity of the pruning regime. Trees were re-pruned in subsequent years to remove stronger up-turning shoots and any vigorous lateral shoots by removing 25 or 50% of the previous year's growth. Thereafter, severely pruned trees were maintained at a height of 2 m.

4.2 Cone Induction

Under natural conditions, western hemlock can remain in its vegetative growth phase for 20–30 years (Puritch 1972; Owens and Molder 1984). However, extensive flowering research under controlled cultural and environmental conditions has shown that western hemlock responds well to several cone induction procedures. Most of these involve a mixture of gibberellins known as GA4/7. This mixture of gibberellins has been used in conjunction with treatments that include: fertilizer (Pollard and Portlock 1983), temperature (Pollard and Portlock 1981a, 1981b), water stress (Ross et al. 1981; Brix and Portlock 1982), and photoperiod (Pollard and Portlock 1984). The treatments have been tested alone and in various combinations. Girdling as an induction technique has not been used extensively. In British Columbia, Parkinson (1989) tested girdling operationally and found that it did not improve either pollen or seed cone production, but Rayonier's Washington State seed orchard has used girdling successfully (C. Cartwright, pers. comm.).

Much of the success of induction treatments has been attributed to timing of treatments in relationship to seasonal patterns of reproductive bud development. A summary of these patterns is shown in Appendix 1, Figure 20. (See also Owens and Molder 1984.)

Both foliar spray and stem injection techniques for GA4/7 application are available. The latter is more commonly used at present. Treatments generally begin at vegetative bud burst, and foliar spray treatments continue for 6 weeks. Owens and Colangeli (1989) suggest that spraying should start at the earlier stage of vegetative bud swell. This is considerably before the start of cone differentiation (Owens and Molder 1974). In another timing of application study, Harrison and Owens (1992) showed that GA4/7 spray application had its greatest effect on seed and pollen cone production when applications began before the onset of cone bud differentiation. Earlier treatments also led to increased cone bud production (especially pollen buds) but the optimal increase occurred at the time of vegetative bud burst.

The techniques for foliar spray are outlined by Ross (1989) and Harrison and Owens (1992). Typically, about 200 mg/L GA4/7 is used in a 0.05% solution (weight/volume) of the aqueous **surfactant** Aromox C12/W. The GA is mixed with the Aromox first and then diluted to the final concentration with warm water. The solution is applied to the foliage to run off followed by a fresh water wash the next day (to reduce the **phytotoxic** effects of the surfactant). Treatments are applied weekly for 4–6 weeks beginning around vegetative bud burst.

Stem injection of GA4/7 is a one-time treatment. Normally, the concentration of GA4/7 applied is adjusted to tree size (stem diameter or crown diameter). Harrison and Owens (1992) used 4 mg of GA4/7 in 0.1 mL of 95% ethanol for 7- to 8-year-old ramets. The solution is pipetted into a 4–5 mm hole drilled about 2 cm deep into the stem at a downward angle about 25 cm above ground level. For larger trees, the dose of GA4/7 can be increased proportional to stem diameter. The same concentration can be used but the number of holes is increased by one for every 5 cm of stem diameter.

There are some problems arising with the supply of GA4/7. Currently our only supplier is Abbott Laboratories (1401 Sheridan Road, North Chicago, Ill. 60064-4000, USA). They have recently formulated the gibberellin mixture into a proprietary product called ProCone™. The active ingredient of ProCone™ is GA4/7 at a concentration of 4% but we do not know what the inert ingredients (carriers) are. Thus, the given protocol for flower induction in western hemlock will only be useful as long as crystalline GA4/7 is in stock. If ProCone™ is to be a meaningful substitute, we will have to test its induction efficacy before recommending it as an operational substitute. Furthermore, we will have to deal with Canadian import laws governing the import of GA4/7 as a registered pesticide in the United States.

4.3 Pollen Management

Because pollen is relatively easily to handle and contributes 50% of the genetic worth of seed lots, effectively managing pollen offers orchard managers a powerful tool to manipulate the genetic structure of their seed lots. Managing pollen is also essential for breeders to complete mating designs in an orderly and timely fashion. Pollen management refers to the collection, extraction, storage, testing, and re-application of pollen. Pollen can be applied either fresh or after storage. Controlled pollination (CP) is the application of small quantities of selected pollen while eliminating other sources of pollen. Supplemental pollination (SP) (also referred to as supplemental mass pollination or SMP) is the application of large volumes of pollen under open pollination conditions.

The extent to which any artificial pollination program is successful depends largely on the viability of the applied pollen. Therefore, maintaining pollen viability during handling and storage is the principal goal of pollen management, and viability testing is an essential part of the process.

Pollen is a living organism, and its activity *ex situ* is controlled primarily by its moisture content and temperature. When pollen is shed from its cone, it is particularly vulnerable to high humidity (>60%) and high temperatures (>30° C). Pollen development leading up to pollen shed can also be adversely affected by environment.

When dealing with pollen it is important to be able to recognize stages of pollen development in the field and to understand the biological significance of these stages. A brief account of these stages is given in the following paragraph, and there is a more detailed presentation in Appendix 1.

Pollen buds are initiated and differentiated the year prior to shedding and over winter at the **pollen mother cell** stage. The pollen sacs (**microsporangia**) borne on bract-like structures (**microsporophylls**) begin to swell and break their bud scales in late winter. This process is very temperature-dependent and occurs from February to early March. The first appearance of the pollen cone as it breaks its bud scales is the purple cluster of microsporophylls. Maturation of the **microspore** occurs during the elongation of the cone and is complete when the cone axis has completely emerged from the bud scales. Pollen shed will occur 1–2 weeks after the microsporophylls begin to separate from the cones. On the coast this begins towards the end of March to early April.

4.3.1 Collection/Extraction Pollen management requires a sound knowledge of both the **phenology** and **cytology** of pollen development. A summary of this development in Appendix 1 is based on the work of Colangeli and Owen (1988). They use nine stages of pollen cone development.

For our purposes we find the following six stages adequate:

Stage 1 Dormant bud; pollen mother cells (Plate 1A)
Stage 2 Initial pollen cone swelling; meiotic cells (Plate 1B)
Stage 3 Bud burst; one-celled pollen (Plates 1C and D)
Stage 4 Elongation of the pollen cone axis; one-celled pollen to four-celled pollen (Plates 2A and B)
Stage 5 Pollen shed; five-celled pollen (Plate 2C)
Stage 6 Spent pollen cone (Plate 2D)

Pollen cones can be picked at stage 4 but it is always better to wait until the first signs of pollen shed begin. We pick the pollen bud rosette and dry the buds at 30° C at an average humidity of 30%. This procedure is consistent for several other conifer species (e.g., Douglas-fir, interior spruce and lodgepole pine). Most of our initial experiments with pollen drying were done with a dryer designed to control temperature and monitor both temperature and humidity during the drying cycle. This system, described in Webber and Painter (1996), can reduce about 25–30 L of wet buds to a moisture content between 5 and 10% of the extracted pollen in about 36–48 hours. The only difference between drying western hemlock and Douglas-fir pollen is the mesh size for the bags used to contain the drying pollen buds and shedding pollen. The diameter of western hemlock pollen is about 60 microns, ranging between 59 and 71 microns (Owens and Simpson 1986). The pore size of the extraction bags is about 45 microns. Average yield is about 45 mg pollen per mL of buds, or approximately 10% (volume of dried pollen buds to volume of pollen extracted).

Although good quantities of pollen can be picked in reasonable time, it may not be cost-effective for large scale pollination programs. A good alternative, vacuum collection of the shedding pollen, may be considered. We have successfully used a small portable generator and a backpack vacuum with good success. We replace the vacuum bag with a mesh screen (silk screen material) with a pore size of about 45 microns. Obviously the problems of supplying power to a field operated machine and vacuuming shedding pollen under wet or high humidity weather is a detraction. However, the alternative of not being able to produce sufficient quantities of high gain seed lots may be the incentive needed to develop cost-effective pollen collection systems.

The other problem with vacuum collection of shedding pollen is drying extracted pollen. It is far more easy to dry pollen buds and then extract pollen at a moisture content suitable for storage. However, when shed pollen is collected and needs further drying, then one of the following techniques may be employed (details provided in Webber and Painter 1996). For small lots (<10 mL), we use a desiccator cabinet with a fully charged (dry) desiccant. Pollen is spread thinly and evenly in a 10 cm diameter Petri dish and exposed to the desiccant until moisture content is in the desired range (5–10%). Rate of drying will depend on the initial pollen moisture content, the size and numbers of lots, and the size and charge of the desiccator chamber. For a chamber about 30 x 40 x 50 cm and a volume of desiccant of about 500 mL, about 15 Petri dishes with about 150 mL of pollen can be dried to 5–10% MC in a few hours.

For drying larger volumes of pollen (few hundred mL to several L), a drying column, such as described in Webber and Painter (1996; p. 14), can be used. These columns are not commercially available, so their use will be limited to those capable of building one. If this cannot be done, then drying small

Photographic Plate 1
Pollen cone development; stages 1, 2, and 3

A. Stage 1 *Dormant buds; females in terminal positions and males in axial positions.*

B. Stage 2 *Initial pollen cone swelling due to meiotic activity.*

C. Stage 3 *Bud burst resulting from meiotic activity and cone axis elongation.*

D. Late Stage 3 *Bud burst.*

Photographic Plate 2
Pollen cone development; stages 4, 5, and 6

A. Early Stage 4 *Maturing pollen cone resulting from elongation of the pollen cone axis, separation of the microsporophylls, and pollen maturation.*

B. Late Stage 4 *Mature pollen cone; pollen cone can be picked.*

C. Stage 5 *Shedding pollen cone.*

D. Stage 6 *Spent pollen cone.*

batches in the desiccator is still possible. A third but less recommended option is drying in a warm room. Pollen is spread out on paper sheets and left exposed to the room environment until drying is complete. The room should be kept at 25–30° C and the humidity reduced to about 35–40 % RH. Be careful to avoid exposing the pollen to both high temperatures (30° C) and high humidity (70 % RH). Under these conditions, pollen viability can deteriorate quickly.

It is also possible to accelerate pollen development in western hemlock. Colangeli and Owens (1991) placed cut branches into water at room temperature. This technique was effective only if the buds were already at an advanced stage of development. Buds that had elongated 50% beyond the bud scales produced normal yields and viability. Buds forced before this stage produced less pollen and increased the number of abnormally developed pollen grains and lower seed yields in field pollination trials. Accelerated pollen development in potted western hemlock can also be accomplished by simply bringing potted stock into a warm shelter house (J.E. Webber, unpublished data). This is a more effective treatment because the stage of pollen development was not important. Pollen shed occurred about 10–14 days sooner than their outdoor counterparts and the pollen was the same in both yields and fertility potential.

4.3.2 Storage The importance of pollen moisture content on retaining viability has been stressed. If pollen is to be used soon after collection (e.g., for pollination in the same year) then pollen moisture content is less important. However, if pollen is to be stored for any length of time (more than 4 weeks), it is important to know the pollen moisture content before storing. The other two important factors affecting pollen fertility in storage are storage temperature and storage atmosphere. Main factor effects (pollen moisture content, storage temperature, and storage atmosphere) on 1- and 3-year storage of western hemlock pollen is shown in Figure 1. The interaction of these main factor effects for 1- and 3-year pollen storage are shown in Figures 2 and 3, respectively.

The results for 1- and 3-year storage trials for western hemlock are similar to those of most other conifers tested. Pollen reduced to a moisture content below 10% and stored in a container with the air removed (vacuum) at standard freezer temperatures (-25° C) retains fertility, expressed as mean filled seed per cone, for the 3 years tested.

Based on data presented in Figures 1, 2, and 3, we recommend that western hemlock be stored in evacuated containers (no air) with a pollen moisture content not exceeding 10% (preferably between 4 and 8% MC). The lowest freezer temperature we tested was -25° C (operating temperature for a normal commercial freezer). If hemlock behaves similarly to other conifer species, storage at temperatures lower than -25° C can be recommended. Data shown in Figures 2 and 3 suggest that western hemlock pollen could be successfully stored at +5° C for 3 years if air is removed from the sealed container. However, where a freezer is available, pollen should still be stored in evacuated containers at freezer temperatures.

4.3.3 Pollen viability tests The extent to which any applied pollination program is successful depends principally on the viability of the pollen. Therefore, viability testing is a core step of any pollen application program. Laboratory viability tests are available to test potential fertility in most conifer species, including western hemlock. The procedures for hemlock

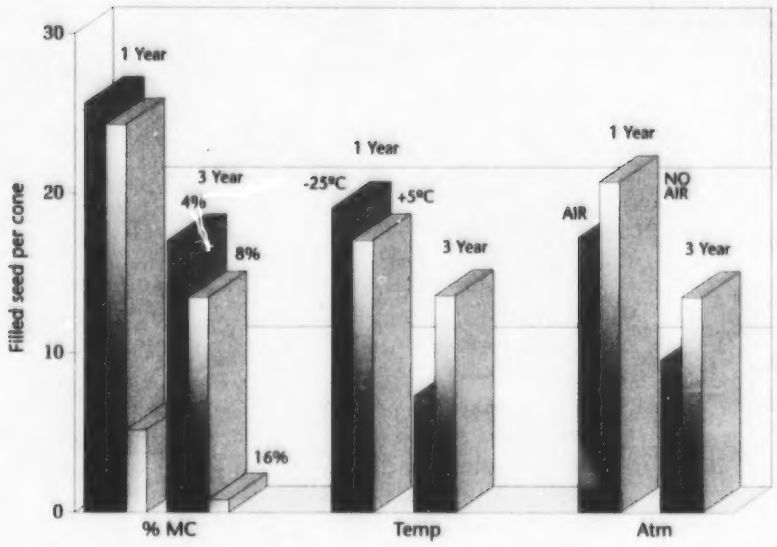

FIGURE 1 **Effects of moisture content, temperature, and atmosphere on pollen storage — 1 and 3 years.**
Main factor effects of pollen moisture content, storage temperature, and storage atmosphere on fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 1 and 3 years.

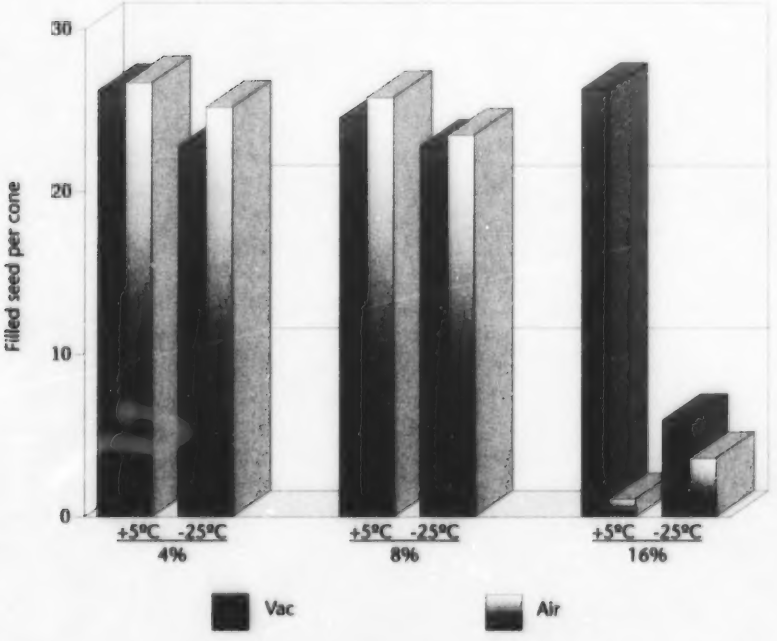

FIGURE 2 **Interaction between moisture content, temperature, and atmosphere on pollen storage — 1 year.**
The interaction between pollen moisture content, storage temperature, and storage atmosphere on the fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 1 year.

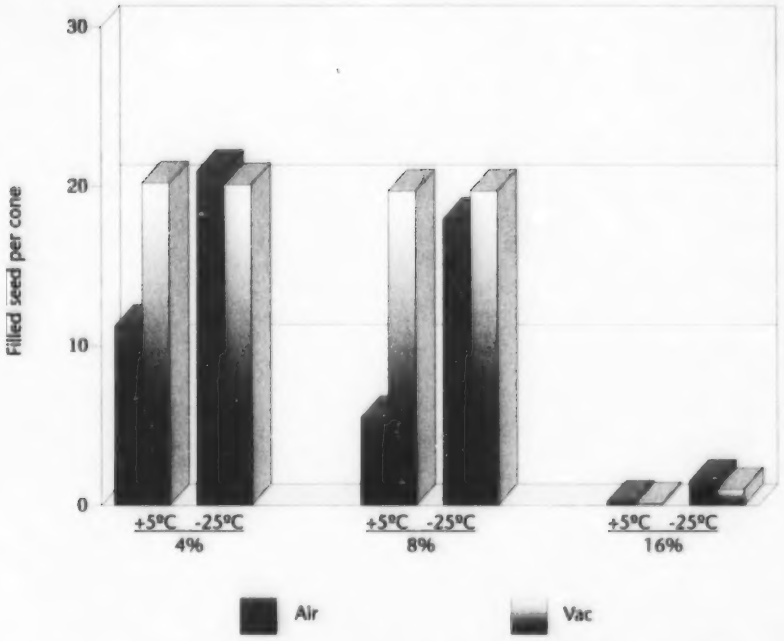

FIGURE 3 Interaction between moisture content, temperature, and atmosphere on pollen storage — 3 years.
The interaction between pollen moisture content, storage temperature, and storage atmosphere on the fertility (expressed as mean filled seed per cone) of western hemlock pollen stored for 3 years.

are adopted primarily from those developed for Douglas-fir, which are summarized in Webber and Bonnet-Masimbert (1993) and Webber and Painter (1996).

The procedures we developed can estimate pollen viability, relative vigour (rate of pollen tube growth), and potential fertility. These tests were developed primarily to estimate the effects of storage on pollen fertility. However, they are also useful for estimating potential fertility of pollen at all stages of handling (e.g., pollen collection and extraction), as well as estimating the yearly variation in pollen viability attributed to environmental effects.

Another important application of viability tests is in formulating specific pollen mixes for improving the genetic worth of production seed lots. Paternal success of individual lots within the mix is affected by its viability. If any particular pollen lot has a significantly lower fertility potential, then the contribution from that lot will be less or not realized at all. Previously, we recommended formulations of pollen mixes with no fewer than 15–20 lots. In this way, probability would dictate whether a low viability pollen lot is in competition with a higher viability lot. More recently, Stoehr et al. (1999) showed that formulating pollen poly-mixes on the basis of viability rather than of volume is an effective way to balance paternal contribution and therefore improve effective population size (Ne).

Under open pollination conditions, where competition from high viability pollen occurs, the estimates of potential parental contribution from pollen lots with a low viability may not be realized. The pollination mechanism (see Appendix 1) of western hemlock is such that pollen competition could be a more important factor than in other species. Because pollen tubes must grow over relatively long distances to reach the nucellus, any reduction in a pollen

lot's vigour will make it less competitive and less likely to contribute to the genetic worth of the seed lot.

We have not studied the pollination mechanism of western hemlock as a factor in manipulating the genetic worth of seed lots, especially where pollen lots of varying levels of viability are pla. d in competition for fertilizing. We do not recommend that low viability pollen lots be used. Specific pollen viability values, below which low viability pollen lots should not be used where competition will occur (e.g., under open pollination conditions), are offered in the subsection, Assay response and fertility (p. 25).

There are many types of laboratory assays to indicate pollen viability but three are most useful for the conifers we have tested. These are:

1. respiration: a measure of oxygen uptake under controlled conditions
2. conductivity: a measure of the conductivity of an aqueous pollen leachate, and
3. germination: a measure of pollen grain growth under controlled conditions.

The following five subsections contain a discussion on pollen preconditioning, which is a necessary first step for testing most conifer pollen lots, a general discussion of each of the three assays, and a final section on the correlation of assay response to field fertility. Additional details on each of these five topics are given in Appendix 2.

Pollen preconditioning When pollen is taken from storage it is in a dormant, dehydrated state, and, before metabolic activity is restored, uptake of water is essential. Imbibition of water depends to a large extent on the osmotic status of the pollen grain and its surrounding environment. Under natural conditions, conifer pollen is shed in a hydrated state, and further water uptake to complete germination and tube growth readily takes place within the micropyle. The same is true for most conifer pollen being tested for viability. Pre-hydration just prior to the assay improves both the assay response and its relationship to field fertility.

The effect of pre-hydration on assay response for western hemlock pollen is shown for each of the three assays described below. For respiration and germination procedures, pre-hydration did not improve assay response or the relationship to field fertility. For conductivity, however, pre-hydrating pollen at 100% RH at 20° C for 16 hours significantly improves the correlation coefficient (r), which is a measure of the strength of the relationship between assay response and seed yields. The proportion of variability in seed yields explained by the assay response is determined by calculating the coefficient of determination (r^2).

The actual time to hydrate varies by species. For western hemlock, pollen moisture content will rise from a dehydrated state of about 8% to about 25% in about 16–18 hours under an atmosphere of 100% RH at 20° C. Figure 4 shows the relationship between pollen moisture content and exposure time to 100% RH at 20° C for two good and two poor viability pollen lots. Where pre-hydration is used prior to assay, we recommend exposure for 16 hours (overnight) at 100% RH and 20° C.

Conductivity Conductivity is a direct measure of pollen membrane stability. When pollen is dehydrated, pollen membranes lose their structural integrity and viability. The extent of this effect depends on the species and the level of moisture reduction. As pollen moisture content is reduced, the membranes become more porous, resulting in reduced membrane activity

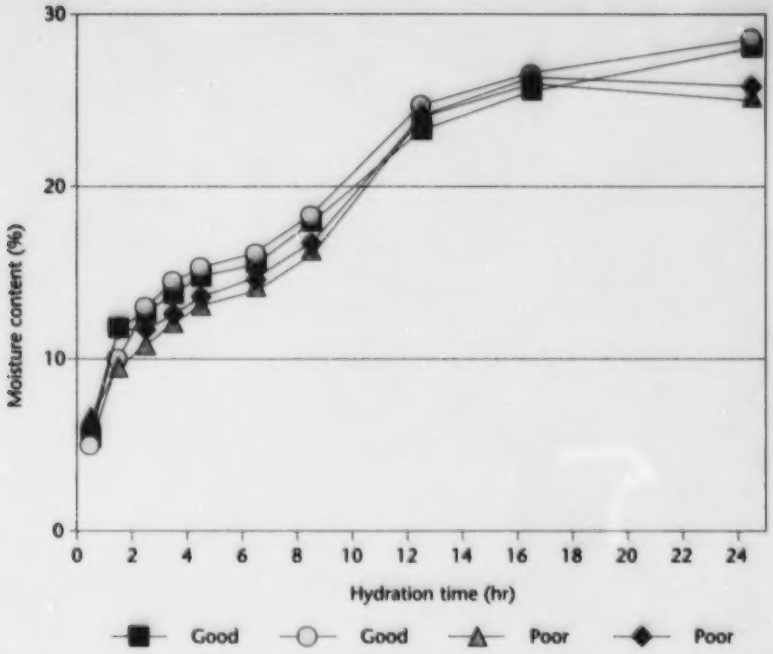

FIGURE 4 Pollen moisture content and hydration.
The relationship between pollen moisture content and hydration interval for two good and two poor viability western hemlock pollen lots.

and an increase in pollen cellular content that can be leached. Since this leachate carries an electrical charge, simple conductivity measurements of the leachate provide a direct estimate of membrane damage and reduction in viability.

The procedure is relatively simple and the assay can be completed in less than 2 hours. However, as for all laboratory assays, more reliable estimates of potential fertility can be made if conductivity is assessed with either germination or respiration results.

Conductivity results can be obtained by simply leaching the pollen in cold water with constant agitation and measuring the electrical conductivity of the leachate. However, variability in the total leached material between pollen lots does occur and can be accounted for by including a hot leachate step. Thus, two analyses are used: cold conductivity and hot conductivity. Percent conductivity is then calculated as the ratio of cold leachate to hot leachate. In most cases, regression coefficients (r) for percent conductivity and filled seed per cone are higher than between cold conductivity and filled seed per cone (Webber and Bonnet-Masimbert 1993; unpublished data for western hemlock).

Both prior hydration and leaching time will affect the amount of leachate and therefore the conductivity results. Figure 5 shows the effect of pollen moisture content on cold conductivity for two good and two poor western hemlock pollen lots. As moisture content rises, cold conductivity values drop for both good and poor viability lots, although to a lesser extent for the poor lots.

The detailed procedures for estimating both cold and hot conductivity in western hemlock pollen are given in Appendix 2. The relationship between

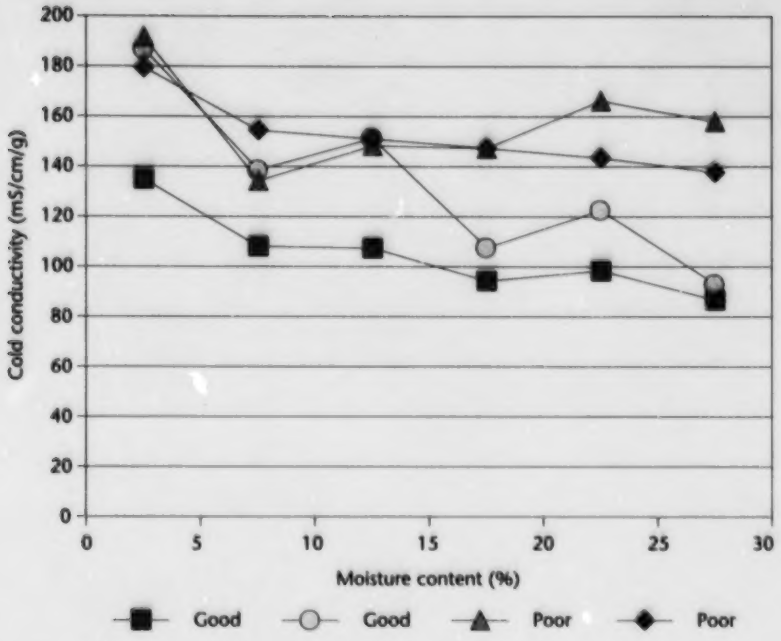

FIGURE 5 Pollen moisture content effects on cold conductivity.
The effect of hydrating good and poor viability western hemlock pollen lots to various moisture contents on cold conductivity.

percent conductivity assay response and field fertility is described in the subsection on Assay response and fertility and shown graphically in Figure 15.

Germination Pollen grains of most conifer species will germinate and grow a pollen tube when cultured in a suitable medium. Of all the conifer species, western hemlock forms the longest tubes both under natural conditions and in culture. This unique feature of western hemlock pollen and its mechanism for being captured is explained further in Appendix 1. Although germination response to laboratory and field conditions may be different, the techniques we have developed for assessing laboratory response are still a good measure of a pollen lot's potential fertility.

Most conifer pollen grains can readily germinate in aqueous solutions with little or no added nutrition. However, in water alone germination is often incomplete. Tube lengths are shorter and in many cases pollen grains will burst if the osmotic potential of the pollen grain and the solution are considerably different. Mature pollen grains normally carry sufficient food reserves (free sugars, starch, lipids, and protein) to enable them to be independent identities from the time of pollen shedding through germination and early tube growth. In general, conifer pollen can be cultured on relatively simple media. Several factors affect pollen response in culture, among which hydration state and medium composition are the most important.

Photographic Plate 3 Classes of western hemlock pollen germination

Germination of western hemlock pollen is classified into three categories (see Figure 6):

Class 1 elongating pollen tube is >2x original pollen grain diameter.
Class 2 elongating pollen tube is <2x original pollen grain diameter.
Class 3 grain is hydrated (original diameter) but no pollen tube is present.

The total number of pollen grains scored in each of Class 1 and 2 determines the overall germination rate expressed as a percentage of Class 1+2 grains to total grains counted.

The effect of hydration state and media composition on germination of western hemlock pollen can be seen in the following figures. Based on laboratory tests, pre-hydrating pollen for germination assay is not necessary for germinating western hemlock pollen since the correlation coefficients (r) did not improve the relationship between assay response and field fertility (see subsection on Assay response and fertility)

The germination medium for western hemlock started with the results of Ho and Sziklai (1971) who used an aqueous solution of Brewbaker and Kwack's (1963) solution. However, by adding other components to this medium, we improved both assay time and response. The media components we tested were: (1) Brewbaker's solution (Brewbaker and Kwack 1963), (2) polyethylene glycol (PEG, molecular weight 4000) as an osmoticum, and (3) sucrose as either an osmoticum or carbon source. These components were tested in either an aqueous solution or a semi-solid agar medium.

The effects of three concentrations of sucrose and PEG in an aqueous solution, with and without Brewbaker's solution, for hydrated and unhydrated western hemlock pollen lots, are shown in Figures 6, 7, 8, and 9. Figure 10 shows the effect of three concentrations of agar (solid medium) without any added components. Note that the response of Class 1 grains is better with all three agar concentrations alone than with any other aqueous media types. We did try various combinations of agar with sucrose and

PEG and Brewbaker's solution. Our maximum germination response (Class 1+2 grains) was obtained with a medium containing 0.25% agar, 5% sucrose, and 10% Brewbaker's solution (Figure 11).

Pre-hydration of pollen improved assay response for aqueous solutions of media (see Figures 6, 7, 8, and 9). However, for the solid media containing agar, the effect of pre-hydration is less important (see subsection on Assay response and fertility). It is clear that media affects assay response. The 0.25% agar containing 10% sucrose and 10% Brewbaker's solution yielded the highest response of Class 1 gains under the conditions we used. However, it may well be that other media types are better. For now, the germination techniques described in Appendix 2 will adequately indicate the pollen lot's potential fertility. The relationship between germination assay response as well as conductivity and respiration to actual field fertility (filled seeds per cone) is described in the subsection, Assay response and fertility below and is shown graphically in Figure 13.

Respiration Respiration is a measure of oxygen uptake that provides an overall estimate of the metabolic status of pollen. The laboratory procedures used to measure oxygen uptake by pollen are straightforward but require rather sophisticated equipment. Because of the technical requirements to maintain, calibrate, and operate the equipment, respiration analysis may not be suitable for operational use. However, from extensive experience with other species, the rate of oxygen uptake has consistently given us the best estimate of pollen fertility. Furthermore, the actual assay procedure is quick, and estimates of pollen fertility can be made in less than 10 minutes. Specific details on determining the oxygen uptake of pollen is given in Appendix 2.

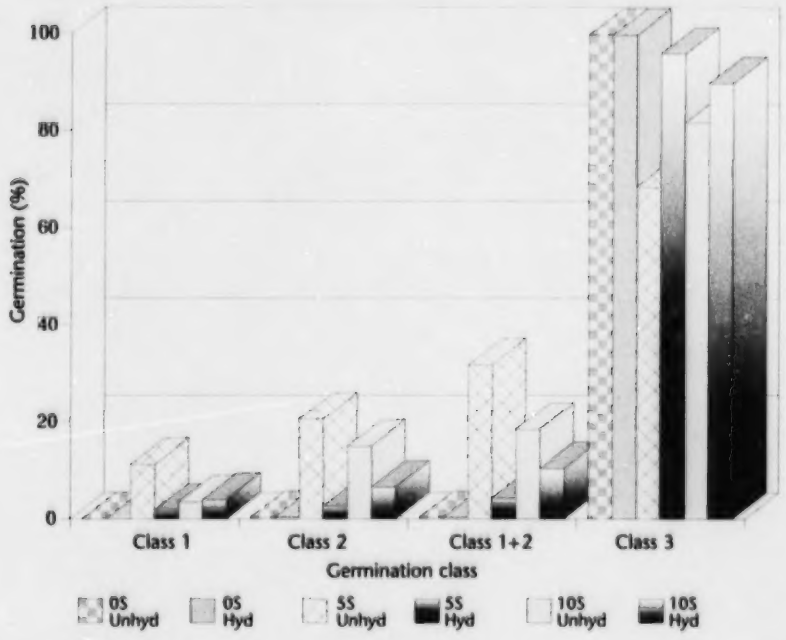

FIGURE 6 **Sucrose concentration and hydration effects on germination.** *The effect of three concentrations (0, 5, and 10%) of sucrose (S) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.*

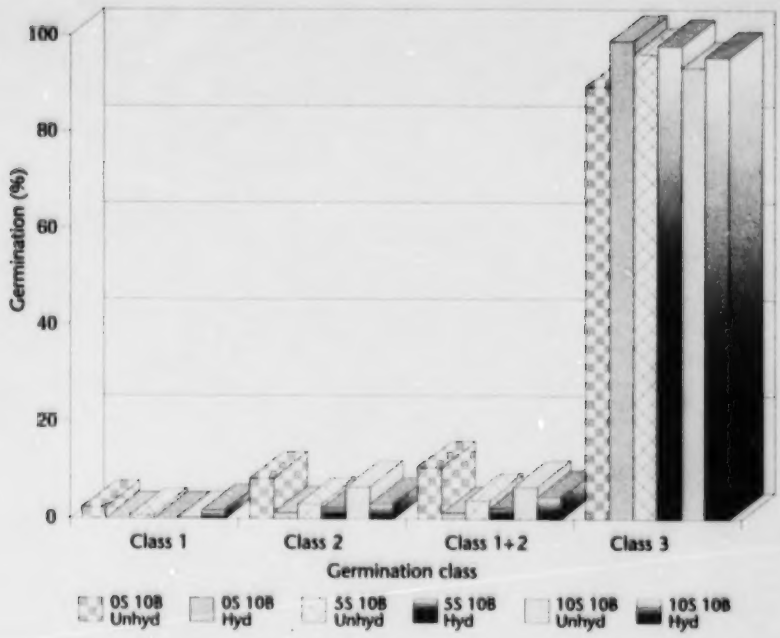

FIGURE 7 **Sucrose concentration, hydration, and Brewbaker's solution effects on germination.**
The effect of three concentrations (0, 5, and 10%) of sucrose (S) with 10% Brewbaker's solution (B) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

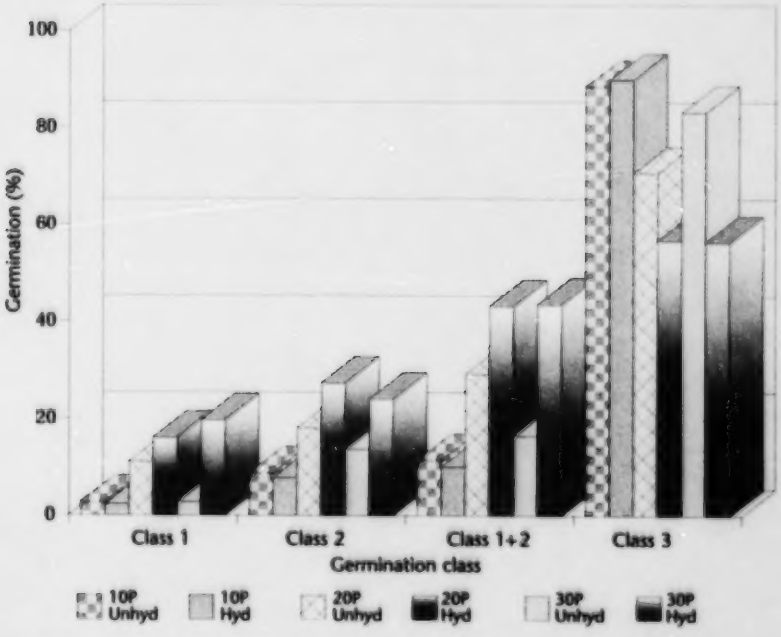

FIGURE 8 **Polyethylene glycol and hydration effects on germination.**
The effect of three concentrations (10, 20, and 30%) of polyethylene glycol (P) on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

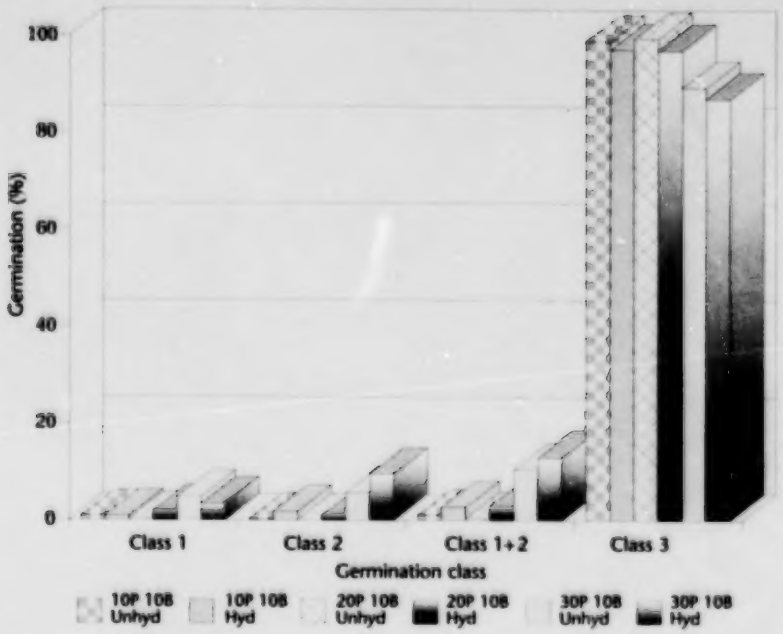

FIGURE 9 **Polyethylene glycol, hydration, and Brewbaker's solution effects on germination.**
The effect of three concentrations (10, 20, and 30%) of polyethylene glycol (P) with 10% Brewbaker's solution (B) on the germination of hydrated and unhydrated western hemlock pollen.

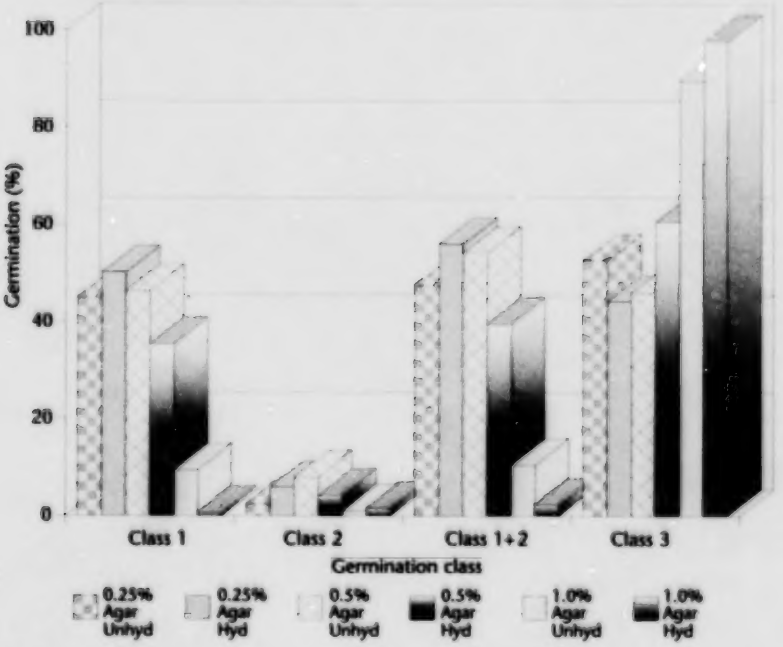

FIGURE 10 **Agar and hydration effects on germination.**
The effect of three concentrations (0.25, 0.5, and 1%) of agar on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains.

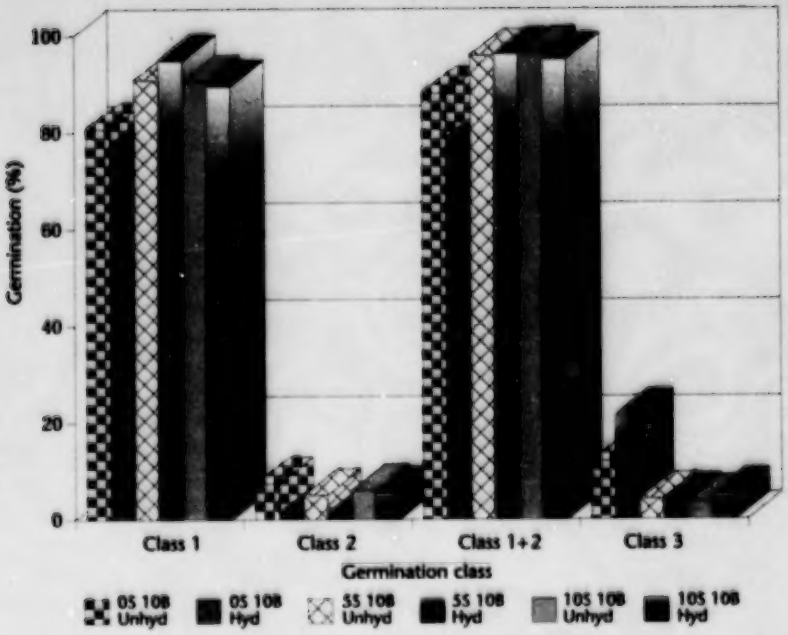

FIGURE 11 Agar, hydration, and Brewbaker's solution effects on germination. *The effect of three concentrations (0, 5, and 10%) of sucrose (S) with 10% Brewbaker's solution (B) in 0.25% agar on the germination of hydrated and unhydrated western hemlock pollen for Class 1, Class 2, Class 1+2, and Class 3 grains. The recommended medium for germinating western hemlock pollen is 0.25% agar containing 10% Brewbaker's solution and 5% sucrose.*

Figure 12 shows the oxygen uptake for two good and two poor pollen lots each hydrated and dehydrated. The effect of pre-hydration is more apparent for the poor lots than for the good lots. Hydration does improve assay response but its effect on the correlation coefficient (r) is not significant. The relationship between respiration assay response and field fertility (filled seed per cone) is described in the following section and is shown graphically in Figure 14.

Assay response and fertility Laboratory tests of any pollen lot are of little use if they cannot be related to field fertility or competitive ability relative to other lots. The viability assays we use for estimating fertility potential have been correlated to actual fertility using controlled crossing conditions. There are several factors that produce variability in fertility potential and, therefore, effect the ability of a specific assay response to predict potential seed yields. These factors include, but are not necessarily limited to:

- level of competing pollen cloud density,
- timing of pollen application,
- atmospheric conditions,
- variation in the ability of seed cone parent trees to produce yields, and
- pollen and seed cone parent interactions.

For single lots used under controlled crossing conditions, assay response is more meaningful. When a pollen lot is used in a poly-mix its assay

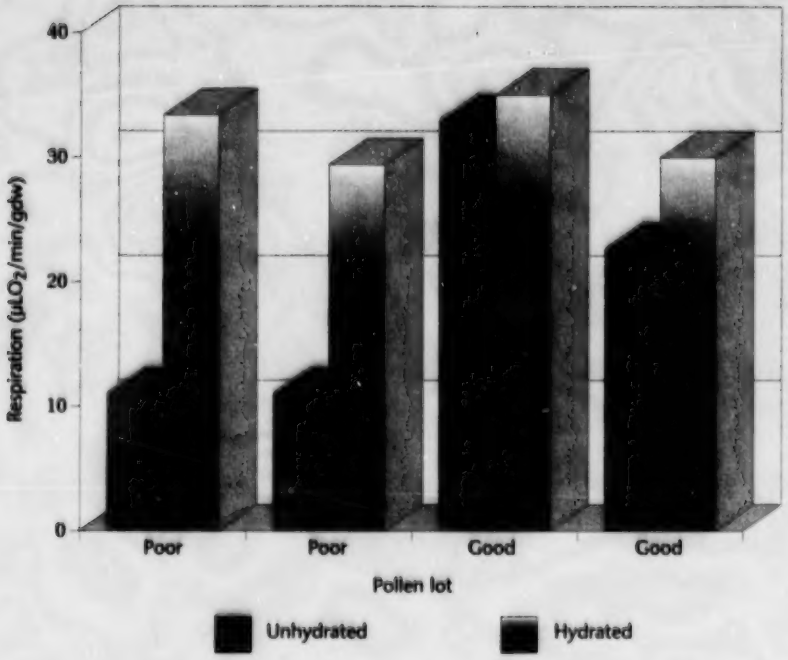

FIGURE 12 **Pollen viability and hydration effects on respiration.** *Oxygen uptake for hydrated and unhydrated poor pollen lots and hydrated and unhydrated good pollen lots.*

response should give a relative ranking of fertility potential for lots used in a pollen mix. For Douglas-fir we have used relative assay responses to formulate the mix, resulting in a better paternal contribution in the resulting seed lot (Stoehr et al. 1999). Even for open pollinated conditions, we strongly recommend testing lots before they are applied. Eventually, we will be able to define limits of pollen viability that can be reliably used to include or exclude any particular pollen lot from a pollen mix.

Figures 13, 14, and 15 show the nonlinear relationship between assay response and field fertility (seed yields) defined as the percent filled seed per cone. The data scatter seen in these figures is typical of the variability associated with pollen testing and field seed yields. The curves generated from these data are also typical. Seed yields are seen to rise as pollen viability (respiration and germination) increases but there is a point (the asymptote) beyond which any further increase in pollen viability is not associated with a corresponding increase in seed yields (see data shown in Figures 13, 14, 15, and 19). The asymptote is generally found around the 50% seed yield value. This is also typical for other species (Webber and Bonnet-Masimbert 1993). On average, our best pollination technique (controlled crossing) produces only 50% of the potential seed available. We are not certain why our filled seed percentage is not higher. The limitation to full yields is more likely related to seed cone development (incomplete ovule formation, which still forms a complete seed coat), cone morphology (limited or restricted access to the ovule), and incomplete pollination than to pollen viability. For our purposes then, 20–25 filled seeds per cone should be considered an operational limit.

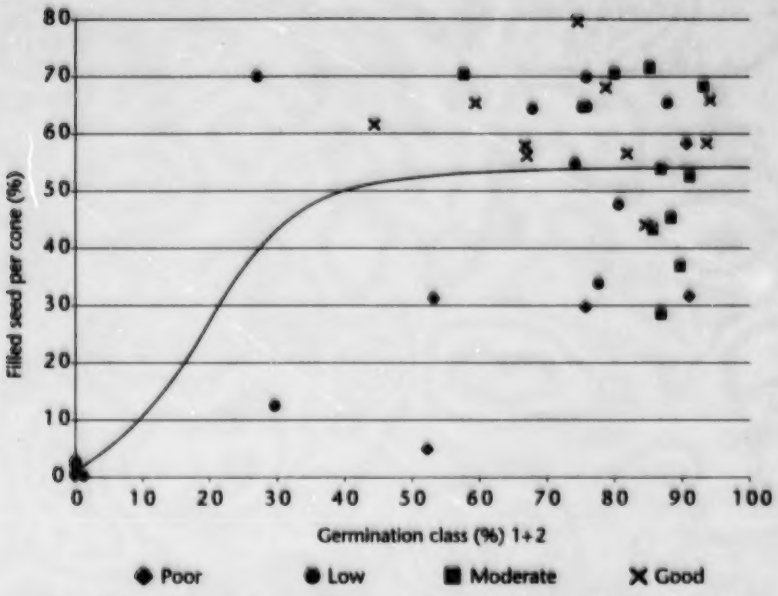

FIGURE 13 **Relationship between filled seed per cone and germination.** *The relationship between percent filled seed per cone and percent germination (Class 1+2 grains) for 40 dehydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the logistic function are a=53.8, b=-0.13, and c=2.61. The coefficient of determination (r^2) is 0.528.*

The curves shown in Figures 13–15 were obtained by classifying pollen lots as poor, low, moderate, and good. We used respiration values to assign pollen lots to these four classes. Actual assay response for percent germination (Class 1+2 grains), percent conductivity (ratio of cold to hot conductivity), and respiration (μL O_2/min/gdw) were determined for each pollen lot from each of the four classes (10 lots per class). Pollen lots were tested both hydrated and dehydrated. Seed yields were determined as average values from controlled crossing using all 40 pollen lots each crossed with seven seed cone parent trees (separate clones). Seed yields were expressed as percent filled seed per cone, which represents the ratio of filled seed per cone divided by potential seed per cone. Potential seed per cone was defined as the number of seeds with a mature seed coat. Regression analyses between assay response and percent filled seed per cone were completed using SAS (1988) nonlinear procedures and various logistic functions. For percent germination and respiration, the form of the logistic function was $y=a/(1+e^{c+bx})$ and for percent conductivity, a hyperbolic function was used of the form $y=ae^{bx}$. Parameters a, b, and c were estimated and then iterated to the best fit by SAS nonlinear procedures.

Figures 13, 14, and 15 show the relationship between percent filled seed per cone and percent germination (Class 1+2), percent conductivity, and respiration (μL O_2/min/gdw), respectively. The values for respiration and germination are shown for dehydrated pollen. The coefficient of determination (r^2) is a direct measure of the variation in seed yields explained by the assay response. For respiration and germination, the r^2 for dehydrated/hydrated pollen are 0.697/0.595 and 0.528/0.523, respectively.

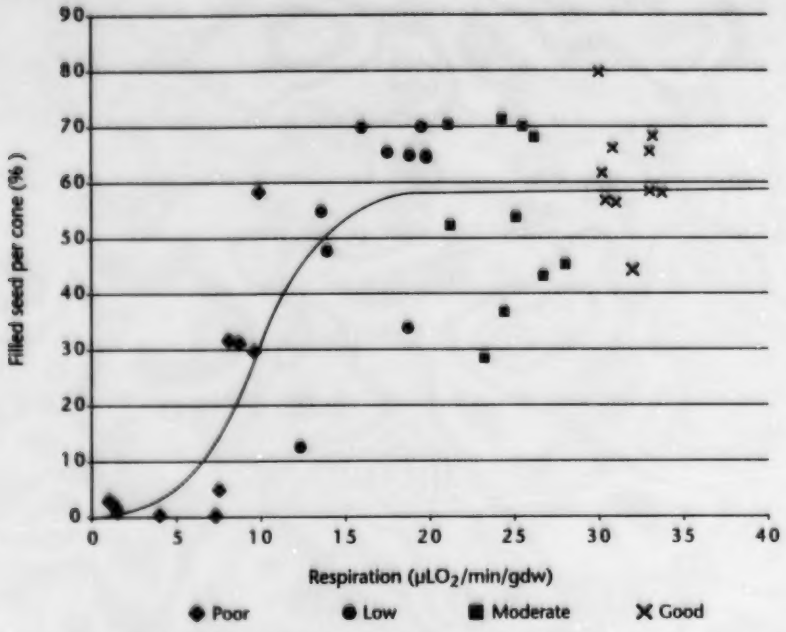

FIGURE 14 **Relationship between filled seed per cone and respiration.**
The relationship between percent filled seed per cone and respiration for 40 dehydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the logistic function are a=58.5, b=-0.45, and c=4.35. The coefficient of determination (r^2) is 0.697.

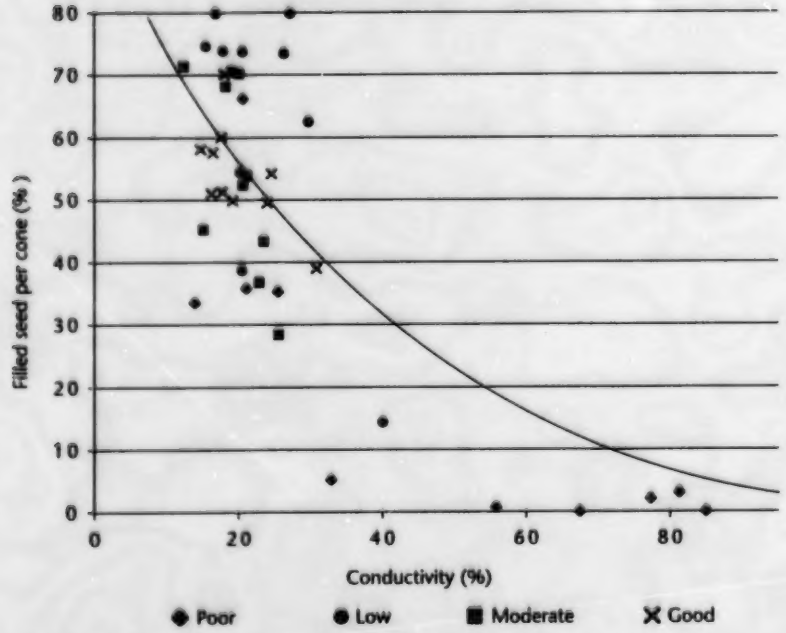

FIGURE 15 **Relationship between filled seed per cone and conductivity.**
The relationship between percent filled seed per cone and percent conductivity for 40 hydrated western hemlock pollen lots segregated into four viability classes (poor=diamond, low=circle, moderate=square, and good=cross). The parameters for the hyperbolic function are a=91.7 and b=-0.02. The coefficient of determination (r^2) is 0.645.

For percent conductivity, however, the r^2 are 0.410 and 0.645 respectively. The improved relationship between percent conductivity and seed yields attributed to hydrating pollen prior to assay is the effect on mending "leaky" pollen membranes.

If we use the 50% potential filled seed per cone as an operational limit for seeds yields in western hemlock, then the corresponding assay response for percent germination, respiration, and percent conductivity are about 45% germination, 15 µL O_2/min/gdw respiration, and 35% conductivity. Values considerably lower than this could still be used for controlled crossing using a single lot pollen parent, but under open pollinated conditions it would be advisable to use only the highest viability lots. We do not know what effect lower viability lots would have on paternal contribution, especially when used in open pollinated conditions. When specific paternity analyses using DNA fingerprint techniques are available, then pollen competition and SP efficacy studies can be completed.

When formulating pollen poly-mixes, two techniques can be considered. First use equal volumes of all pollen lots but use many lots, not less than 12–15. If fewer lots are required then it is possible to use the techniques described by Stoehr et al. (1999), where lot volume is adjusted to viability. Lower viability lots have higher volumes and higher viability lots have lower volumes. Again, when specific molecular markers (DNA fingerprints) are available, these questions can be answered more directly.

4.3.4 Field pollination techniques On average, seed yields for western hemlock are about 20 filled seed per cone (Edwards 1976). Western hemlock has about 20–30 scales per cone (Owens and Molder 1984) suggesting that the seed potential is about 40–60 filled seed per cone (e.g., two ovules are borne on each scale). This potential is seldom realized because about one-third of the scales are not fertile. Other factors affecting seed losses in western hemlock include ovule abortion, insufficient pollination, no fertilization, and embryo degeneration (Colangeli and Owens 1990). From our experience using good pollination technique this should result in about 25 filled seeds per cone. However, considerable variation can occur between clones and within clones, ramets, and cones. This variation results from variability in the timing of pollination, the amount of pollen applied, and from male–female interactions.

To study the optimal time to pollinate western hemlock seed cones, we define six stages of seed cone development. Seed cone buds are initiated in the spring and differentiated by mid-summer. The newly formed dormant bud that overwinters is our first stage of seed cone development (see Plate 1A). Both seed and pollen cones are rather small at this time and they are difficult to distinguish. Identification by dissection is possible but the technique is technically demanding and requires considerable experience.

Starting in February of the second year the seed cone buds begin to enlarge (stage 2) as the result of the onset of meiosis. The cone tips begin to push through the bud scales (stage 3) about 3 weeks before pollination begins (towards the end of March or early April).

The seed cone bracts separate, exposing the ovules (stage 4) at about the same time that the microsporangia dry and split, releasing the pollen. Throughout the pollination period the seed cones are usually purple and remain horizontal or pendant. The ovuliferous scales then enlarge and seal the cone (stage 5). Visually, the seed cone changes from red or purple during receptivity to green at the pendant stage and then brown at maturity. From early bud burst through receptivity and cone closure, the scales and

seed cone axes continue to enlarge and elongate. About 3 weeks after cone closure, the cones become pendant and continue to mature until mid-August when the scales start turning brown and the entire cone begins to loosen (Stage 6). The mature cone is ready for harvest. A more complete summary of seed cone development can be found in Appendix 1.

Western hemlock has a relatively long receptivity period, which is principally regulated by temperature. Optimal receptivity occurs between 2 and 8 days after bud burst (defined to occur when 40–50% of the bud scales are visible) but can still be receptive to pollen up to 12–14 days after bud burst. Figure 16 shows the seed yields for various pollination times beginning at bud burst (day 0) and for 2, 4, 8, and 12 days after bud burst.

While late pollination (e.g., 12 days after bud burst) does produce good seed set, it is likely that earlier pollination may be more effective for enhancing genetic worth. In Douglas-fir (see Webber and Yeh 1987), we have demonstrated that early pollination is preferentially favoured over later pollination (first-on, first-in). This is a result of the unique pollination mechanism of Douglas-fir, which traps early-arriving pollen in the stigmatic hairs surrounding the micropyle.

In western hemlock, the pollination mechanism is quite different. Unlike most other conifer species where the pollen sifts through the space between the seed cone scales and bracts, pollen seldom reaches the micropyle or integument (see Plate 6A; Colangeli and Owens 1989, Appendix 1) but rather adheres to the outer edges of the bracts and (to a lesser extent) scales. The surface of western hemlock pollen is roughly sculptured with spines that adhere to the waxy hair-like surface of the bracts very much analogous to the fastening system of Velcro (see Plate 6B). About 8–9 weeks after bud burst, pollen grains begin to germinate, forming very long pollen tubes that

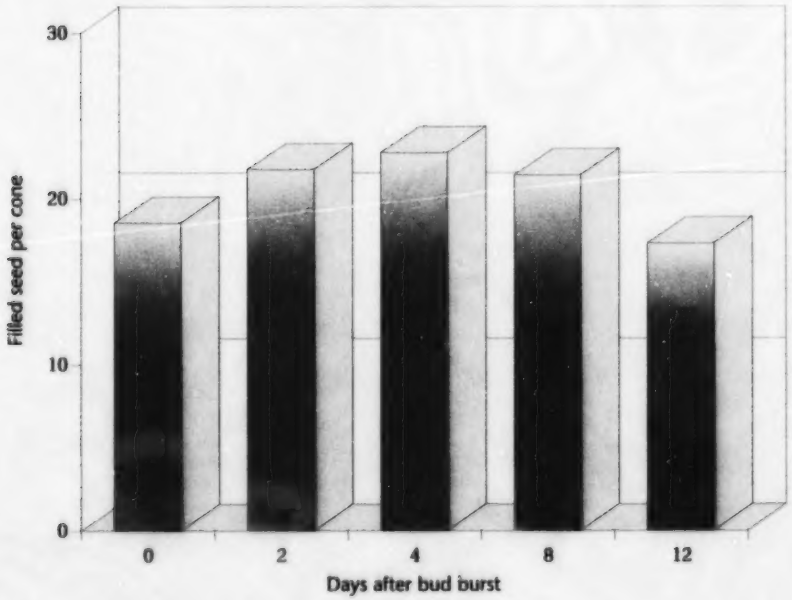

FIGURE 16 **Seed yields and pollination timing.**
The effect of pollinating western hemlock seed cone buds at bud burst (0 days) and at 2, 4, 8, and 12 days beyond bud burst on seed yields (filled seed per cone).

Photographic Plate 4
Seed cone development; stages 2, 3, and 4

A. Stage 2 *Meiotic seed bud; seed cone is beginning to develop. (See Plate 1A for stage 1 dormant seed cones in terminal positions.)*

B. Stage 3 *Seed cone bud burst with 50% of the scales exposed.*

C. Stage 3 *Seed cone bud burst with scales beginning to reflex.*

D. Early Stage 4 *Receptive seed cone bud with more than 50% of the cone bud scales exposed due to cone elongation.*

Photographic Plate 5.
Seed cone development; stages 4, 5 and 6

A. Late Stage 4 *Receptive seed cone bud with all scales fully exposed and reflexing.*

B. Stage 5 *Cone closure. Scale swelling closes the gap between the scale and bract, eliminating further pollen access.*

C. Stage 6 *Mature seed cone bud.*

D. Seed cone development sequence
From very early stage 3 bud burst through receptive seed cone bud at stage 4 to cone closure in stage 5.

grow towards the micropyle and about 1 week later, penetrate the nucellus (see Plate 6C). The important point here is the distance that the pollen tube must cover to reach the nucellus. Because of this particular mechanism, pollen viability and vigour must be important to fertilization success. This suggests that some pollen grains will out-compete others in the race towards fertilization.

We are not certain how to use this pollen mechanism to our advantage when we pollinate western hemlock. Will pollen adhering to the bracts first have an advantage over other pollen grains arriving later? Will high viability pollen out-compete low viability pollen? We have attempted to answer the first question and expect the answer to the second will be yes.

Figure 17 shows the seed yields from a time of pollination experiment in which known good and poor viability pollen lots were applied to receptive seed cone buds. Both poor and good pollen lots were applied first at BB+2 days and then each was followed by either the good or poor lot at 24, 48, and 96 hours. For comparison, seed yields for only the good and poor lots are shown as well as a 50% mixture of good and poor lots.

The data in Figure 17 show indeed that the poor lot resulted in poor seed yields and the good lot produced good seed. The yields from the good lot were not diminished by mixing with 50% poor pollen but, because the poor lot was virtually dead, we cannot make any comments on whether pollen competition could occur. When the good lot was applied first, followed by the poor lot at 24, 48, and 96 hours, there was no effect on seed yield by the later-arriving poor lot. When the poor lot was applied first, followed by the

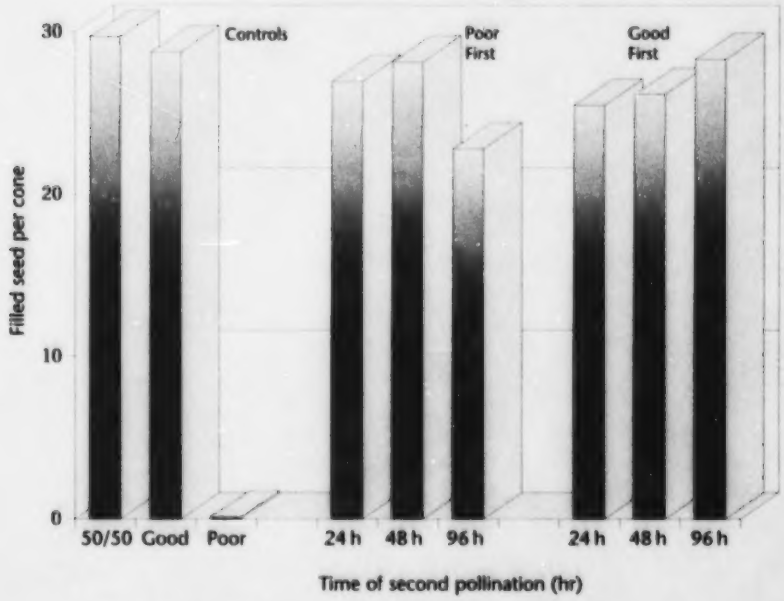

FIGURE 17 **Seed yields and time of arrival for good and poor pollen lots.** *The effect on seed yields of good and poor viability pollen lots applied at BB+2 in western hemlock and then followed with poor and good lots, respectively, applied at 24, 48, and 96 hours after the initial pollination. For comparison, seed yield values for the good and poor lots only are shown as well as the seed yields from a 50% mixture of good and poor lots.*

good lot at 24, 48, and 96 hours, there may have been a slight reduction in seed yields when the good lot was applied at 96 hours but not at 24 or 48 hours. Apparently the time of arrival of pollen in western hemlock does not affect its ability to complete the events leading to fertilization, as we have noticed for Douglas-fir.

4.3.5 Supplemental pollination Supplemental pollination (SP) is the broadcast application of pollen to female receptive seed cone buds that are not isolated from airborne pollen (Bridgwater et al. 1993). It has been successfully used to improve seed yields, to control paternal composition of orchard seed, to introduce specific genotypes into the seed orchard, and to reduce self-fertilization and contamination. Since contamination is not a serious issue in western hemlock orchards, the principal use of SP in hemlock will be the enhancement of seed yields and the improvement of genetic worth and paternal balance.

For western hemlock orchards in British Columbia the species plan does not specify the type of delivery system for genetic gain, and the available options are few. Currently, controlled crossing with bulking up using rooted cuttings is our only other strategy for producing high genetic worth seed lots. However, these bulking up techniques have not been fully implemented operationally, so we will still require effective SP techniques to meet objectives for seed yields and doubling the gain.

Timing and concentration Success of supplemental pollination is very dependent on the quality of applied pollen (viability, vigour, or fertility potential), and how and when the pollen is applied. Most practitioners experienced with supplemental pollination agree that the technique should be more successful than actual studies indicate. Bridgwater et al. (1993) provide a good review of the advantages of supplemental pollination, including the criteria for success. Pollen quality, timing of application, frequency of application, and amount of pollen are all important factors. We have discussed techniques for maintaining pollen fertility at a high level and its application at the optimal stage of seed cone receptivity. We have also mentioned the importance of quantifying pollen cloud density since it too is a principal determinant of supplemental pollination success. Next we will show that SP can improve seed yields.

Figure 18 shows the seed yield results for an SP trial in the Mount Newton western hemlock seed orchard (1994). One ramet from each of eight clones was treated with a pollen poly-mix composed of nine pollen parents. Pollen was applied at BB+4 days, BB+6 days, and BB+8 days beyond seed cone bud burst. Each was a single application and the treated branch was either bagged or left unbagged after application. Since there was virtually no ambient pollen cloud within the orchard, virtually all seed arose from SP application. Timing of application (e.g., the number of days beyond seed cone bud receptivity) was not important and the loss of seed due to leaving the pollinated seed cones exposed (e.g., unbagged) was also not significant. For this test, pollen supply from SP was adequate and losses due to insects were minimal. These results suggest that supplemental pollination for western hemlock is effective, but we have not tested these procedures under heavy pollen cloud competition or when insect populations are more prevalent.

A question we are often asked about SP application is "what is the dilution rate of applied pollen?" We do know that pollen viability can affect seed yield, and the relationship is not linear (see Figures 13, 14, and 15).

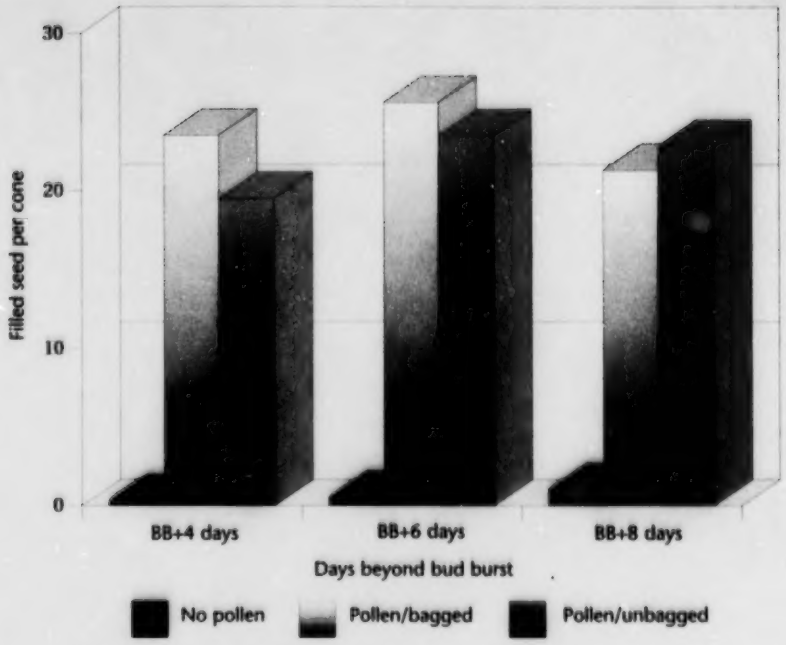

FIGURE 18 Supplemental pollination and seed cone receptivity.
The effect of supplemental pollination at three stages of western hemlock seed cone receptivity for single pollen applications using a nine-pollen poly-mix and comparing treated seed cones left bagged and unbagged.

In general, seed yields rise in a linear fashion as viability increases. However, there is a point (the asymptote) beyond which increasing pollen viability is not associated with a further increase in seed yields. This suggests that high viability pollen lots could be diluted without jeopardizing seed yields. Figure 19 shows this to be true.

In a pollen dilution study, both dead pollen and talc were used to create a series of live/dead ratios. From 0% live, seed yields rose somewhat linearly to about 40–50% dilution rates. Beyond 50% live pollen, there was no corresponding increase in seed yield with an increasing proportion of live pollen. Up to 50% dilution, there were some differences between dead pollen or talc as a diluting agent but we do not feel that these differences were significant.

Although Figure 19 shows seed yield values to be better for talc as the diluting agent at the 50 and 60% dilution, we do not feel that this is biologically significant. For general field pollination, diluting pollen to 40 or 50% may be an effective tool for stretching limited pollen supplies, especially for controlled cross pollination. However, we have not determined the effect of diluting pollen when used under open pollinated conditions or when used in competition with high viability pollen from both known and unknown sources. Again, this pollination technique can be better assessed when we have access to DNA fingerprint analyses.

The biggest question that remains to be answered is "what is the efficacy of SP applied under various pollen cloud densities?" If we do not see an increase in seed yields (e.g., as we see in Figure 18), then we do not know if there is a corresponding increase in genetic worth. This question must wait for the development of unambiguous techniques for determining contributing pollen parents (paternity analyses). We are working on

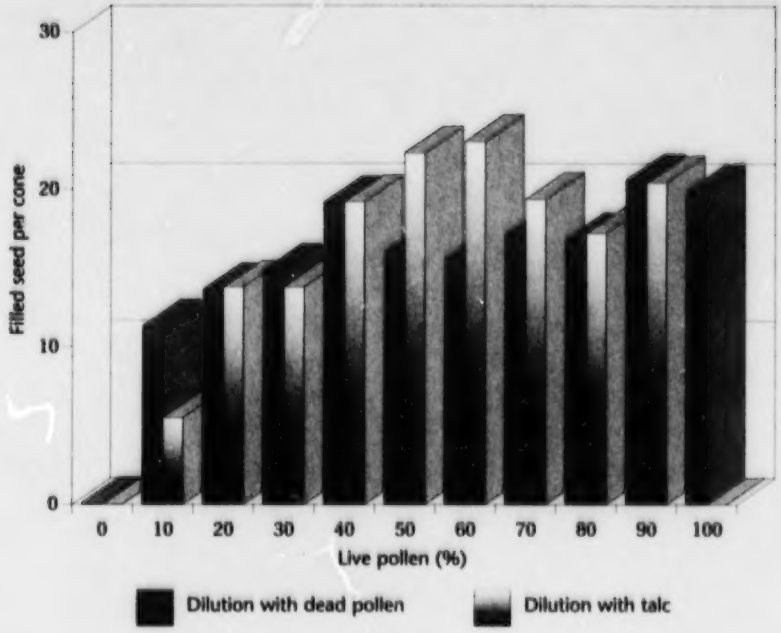

FIGURE 19 Pollen dilution and seed yield.
The effect on seed yields of diluting live western hemlock pollen to specific ratios with either dead pollen or talc powder.

developing specific DNA fingerprinting techniques, and as our diagnostic tools for paternity analyses improve so will our ability to develop SP technique under all orchard conditions improve.

Pollen monitoring We have stressed the effect of competing pollen cloud density (PCD) on optimizing seed yields and genetic worth of western hemlock production crops. Because western hemlock orchards will rely on supplemental pollination to improve yields and genetic worth (at least in the early stages of establishing high gain orchards), then the relationship between competing pollen cloud density and seed yields will be important information to know. Sorensen and Webber (1997) have described this relationship mathematically for western hemlock. Using a log-log model, seed set is seen to rise rapidly with initial increases of PCD, and reaches an asymtope at PCD values that are low compared with maximum values measured under field conditions.

The techniques for capturing airborne pollen and quantifying the captured pollen into cloud density is detailed in the *Douglas-fir Pollen Management Manual* (Webber and Painter 1996). It is strongly recommended that these procedures be used in conjunction with any open pollinated treatments. For controlled crossing and unmanaged orchards, pollen monitoring is not needed. However, for orchards attempting to maximize their genetic worth, knowing the year-to-year variation of within-orchard pollen clouds will help explain the differences observed in SP response for any particular year.

Pollen applicators This manual has not detailed the various types of pollen applicators available. Again, the *Douglas-fir Pollen Management Manual* (Webber and Painter 1996) does provide comprehensive descriptions of the applicator devices we have developed plus others we have tested. There are many types of applicators, each with its own advantages. There are, however, features that make some better than others. It is our experience that applicators that work off compressed air and direct a "blast" of pollen directly at the receptive seed cone are consistently better than those that create only a mist of pollen that is allowed to "float" over the seed cones. Regardless of the way SP pollen is applied, we encourage all users to determine paternal contribution as the best way to guide this developing technique.

The Forest Genetics Council's objectives for western hemlock seedling production requirements up to the year 2007 can be met with current operational seed production capabilities if we:

- develop seed orchard protocols based on field-tested techniques,
- continue to develop supplemental pollination technologies and DNA fingerprint analyses for their assessment, and
- vigorously integrate the new technologies into management of existing and future seed orchards.

What we have learned is summarized in this manual and includes research summaries for cone induction and crown management, and research results for a wide range of pollen management techniques. Although all of these are important, focusing on pollen management, especially supplemental pollination, will enable us to make significant gains immediately.

The most severe limitation to developing technologies for producing seed of the highest genetic worth is determining supplemental pollination efficacy. A major unknown in this process is the effect of competing pollen, and we need to complete trials under various pollen loads to develop supplemental pollination techniques that work for western hemlock.

When these pollination techniques are considered in conjunction with management techniques already developed, we will be able to design orchard systems that will help us meet our target goals for high genetic worth seed lots. In the meantime, if we vigorously utilize recently developed techniques in existing orchards, significant improvement in seed yields and gains in genetic worth are possible.

John Owens and Marje Molder have provided an excellent review of the reproductive biology of western and mountain hemlock (Owens and Molder 1984) with 40 references to additional work. The following summary relies heavily on that review, and there has been no attempt to cite the work every time it was used.

Reproductive potential Western hemlock at lower elevations is generally a good cone and seed producer. On open grown trees the best cone crops form between 25 and 30 years. Individual trees usually produce some cones every year and heavy (collectible) cone crops occur every 3 or 4 years. Cones produce about 30 or 40 seeds each, about half of them being filled.

Growth cycles A schematic of the development of reproductive buds and cones of western hemlock is shown in Figure 20. The schematic is for western hemlock in southern or low elevation regions where activity starts earlier in the spring and continues later into the fall than for trees from northern or high elevation regions.

Figure 20 illustrates the 2-year growth cycle starting from a dormant vegetative bud and ending with a mature seed cone. This schematic shows the flushing of the dormant vegetative bud that occurs in mid-May but no other vegetative development. Vegetative stages not shown in this schematic are flushing that precedes bud swell in late March, rapid shoot elongation in late spring and early summer, and slow shoot elongation through mid-summer.

Axillary bud initiation begins in April, and these axillary buds have the potential of developing into vegetative shoots, pollen cones or seed cones. The dormant vegetative bud shown as the start point of Figure 20 is the second year stage of an axillary, vegetative bud initiated the previous year. Pollen cone development starts in June and seed cone development in July, and both types of cones develop during the fall of the first year.

Pollen development with the associated meiosis in late February and March is the first activity of the second spring. This is followed by seed cone development and pollination in late March and April. Fertilization occurs in late May following female gametophyte development. Embryo development proceeds through the summer, and the seed cones are mature by the end of August.

The following seven sections describe the events outlined in the previous three paragraphs in more detail. Parenthetical references to plates are to the colour plates in the Collection/Extraction (Plates 1 and 2) and Field pollination techniques (Plates 4 and 5) sections of the main text.

Bud differentiation Differentiation of axillary buds occurs after bud scales have been initiated. This normally occurs soon after the rapid elongation stage of the current year's shoot. An axillary bud may (1) initiate leaf primordia to become a vegetative bud, (2) initiate bracts to become a seed cone bud, or (3) initiate microsporophylls to become a pollen cone bud.

Seed cones always form on the terminal buds of lateral vegetative shoots that are 1 year or older. Pollen cone buds most often form on newly initiated lateral buds. Male buds are differentiated first on shorter proximal shoots in the lower crown, followed several weeks later by seed cones typically borne on longer distal shoots in the upper crown. States of

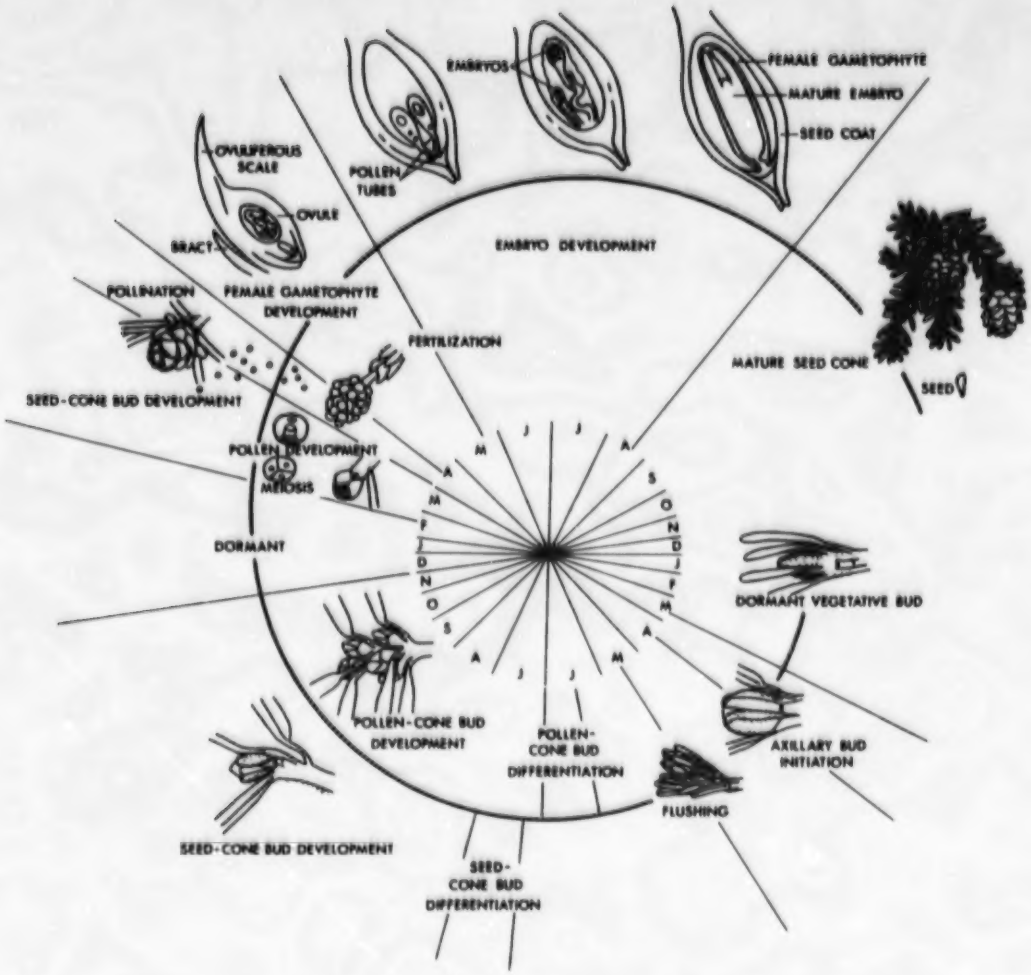

FIGURE 20 Development of reproductive buds and cones in western hemlock.
Schematic of the 2-year cycle from dormant vegetative bud to spent seed cone of western hemlock. (From Owens and Molder 1984, Figure 5.)

differentiation occur during a 2-week period and are only visible microscopically. The methods of early identification of buds are too tedious for routine work.

By summer, buds can usually be distinguished on the basis of external characteristics. Vegetative buds are more pointed than cone buds. Seed cone buds are broader than vegetative buds and the shoot at the base of the seed cone bud bends at a distinct angle from the subtending shoot. Pollen cone buds tend to be more rounded than seed cones but are most easily distinguished by their distribution. Several buds are usually clustered around the base of a short shoot (Plate 1A).

Pollen cone and pollen development After differentiation in the first year, pollen buds overwinter at the pollen mother cell stage (see Ho and Owens 1974). Development resumes the following spring with the onset of **meiosis**, the process by which the **chromosome** number is halved (from 24 to 12). This halving is accomplished by the division of the pollen mother cell into four microspores. This is the first identifiable stage of spring

development (Plate 1B). The pollen sacs (microsporangia) borne on bract-like structures (microsporophylls) begin to swell and break their bud scales from February into early March. The first appearance of the pollen cone, known as bud burst, is the purple cluster of microsporophylls as they break through the bud scales (Plates 1C and D).

The pollen cone continues to elongate and the microspores expand with the accumulation of starch and lipids (Colangeli and Owens 1988). When the microsporophylls begin to separate, the cones are very near shedding (Plate 2A). Maturation of the microspore occurs during the elongation of the cone and is complete when the cone axis has completely emerged from the bud scales (Plate 2B). The microspores go through a series of divisions that produce mature, five-celled pollen grains, and these grains are generally shed a few days after being formed (Plate 2C). On the coast, pollen shed can begin towards the end of March to early April. Empty cones (Plate 2D) may remain on the trees for many months.

The early stages of meiosis and microspore development can be sensitive to severe environmental changes, particularly low temperatures (see Webber and Painter 1996 and references therein).

Anna Colangeli and John Owens (1988a) divided pollen cone development into nine phenological stages and compared these with pollen cytology in field-grown and container-grown western hemlock clones. Phenology proved to be an accurate indicator of cytology. The following table is a simplified summary of their work.

In the main text we use only six pollen cone development stages; our stage 4 correlates with stages 4 through 7 in the table below. For field management purposes the pollen is either mature or not, and we do not need be concerned with the cytological stages being considered by Colangeli and Owens.

TABLE A1.1 Western hemlock pollen cone development
Approximate dates of phenological and cytological events for two groups of western hemlock. (Modified from Colangeli and Owens 1988, Table 1.)

Stage	Date	Phenology	Cytology
1	mid-Jan. to late Feb.	Quiescent bud	PMC to end of meiosis
2	early Feb. to early Mar.	Swollen bud	Tetrads of microspores, Separation of tetrads
3	early Feb. to early Mar.	Bud burst	One-celled pollen
4	late Feb. to mid-Mar.	One-half emerged cone	One-celled pollen, Reserve accumulation
5	late Feb. to mid-Mar.	Three-quarters emerged cone	Two-celled pollen, Reserve accumulation
6	early Mar. to late Mar.	Fully emerged cone	Three- and four-celled pollen
7	late Mar. to early Apr.	Stalk elongation	Four- and five-celled pollen
8	late Mar. to mid Apr.	Pollen shed	Five-celled pollen
9	late Mar. to early Apr.	Empty pollen cone	

Seed cone and seed development The number of vegetative lateral buds initiated varies little from year to year, but their subsequent development varies greatly, with dense crops of both seed cone and pollen cone occurring occasionally. The underlying physiology of this is poorly understood, but much information on the effects of external factors on cone bud differentiation has been collected. For cone production, the important stages of development are the later stages of rapid shoot elongation (Owens and Colangeli 1989; Ross 1989).

Environmental factors that enhance cone bud formation include abundant sunshine, warm temperature, and low rainfall preceding and during the time of cone bud differentiation. Of the many artificial methods of enhancing cone bud formation that have been tried, the most successful for western hemlock involves combined use of gibberellins, nitrate fertilizers, and water stress. Generally, however, gibberellin A_4 and A_7 combined with naturally occurring summer drought seem to be sufficient to induce flowering in hemlock.

After being initiated in the spring, seed cone buds are differentiated by mid-summer. Differentiation consists of the initiation of bracts followed by the formation of ovuliferous scales at the base of the bracts. Ovule primordia are initiated on the larger ovuliferous scales before the end of the first year, making it possible to distinguish the buds visually by the end of the first fall (Plate 1A).

Starting in February of the second year the **nucellus** develops on each ovule. Integument tissue then forms on the end of each ovule; this develops into a short funnel with a wide opening known as the integument tip (Plate 4A). What one sees as the result of these cellular developments is that the seed cone buds enlarge. Bud burst, the emergence of the cone tips through the bud scales, occurs about 3 weeks before pollination begins at the end of March (Plates 4B and C).

Pollination Pollen cone and seed cone development varies markedly with temperature, so pollination can vary significantly in different locations within a given year and in the same location in different years. Pollen and seed cone development can also be non-synchronous. However, in a specific time and place the pollen cone axis elongates and the seed cone emerges from the bud scales in unison. The seed cone bracts separate, exposing the ovules, and the microsporangia dry and split, releasing the pollen. Throughout the pollination period the seed cones are usually purple and remain horizontal or pendant (Plates 4D and 5A).

After pollination in the early spring the pollen adheres to the bract, ovuliferous scale, ovule, or integument tip of the seed cone (Plate 5B). After 5–6 weeks the pollen will germinate on almost any surface within the cone. After germination each pollen grain forms a pollen tube that grows down to each nucellus. The ovuliferous scales then enlarge and seal the cone (Plate 5C).

Ovule development and fertilization Meiosis starts after winter dormancy (i.e., concurrent with pollination) and the cell divisions that are part of this process result in the formation of the female **gamete**. In the next 6-week to 2-month period specialized cells form around the gamete, resulting in the filled seed. The seeds will mature (i.e., the gametes, ovules, and cones will develop fully) without pollen. Visually, the best indicator of seed maturity is the integument and scales.

Photographic Plate 6
Seed cone development and pollination

A. Scanning electron micrograph (SEM) of a fully enlarged ovule (o) in which the integument (i) has elongated, forming a short microphyle (m) (Colangeli and Owens 1989, Figure 14).

B. SEM of pollen (p) entangled in the epicuticular waxes (w) on the bract (Colangeli and Owens 1989, Figure 19).

C. SEM of a seed cone with an ovuliferous scale removed, showing pollen tubes (pt) growing into the microphyle of an ovule (o) (Colangeli and Owens 1989, Figure 28).

There are multiple eggs within an ovule. Several pollen tubes may grow into the nucellus, but no more than one will penetrate each **archegonium**. The body cell that is carried through the pollen tube divides to form two unequal-sized male gametes. The contents of the pollen tube are released into the egg cytoplasm, and the larger male gamete moves through the egg cytoplasm and fuses with the egg nucleus. This constitutes fertilization. If no eggs are fertilized within a female gametophyte, it will degenerate, leaving a normal-appearing but empty seed.

Seed development, cone maturation, seed release, and regeneration The embryo undergoes a short development stage within the archegonium that produces 16 cells and a longer phase that takes place in the female gametophyte tissue. While a seed is maturing, as many as 16 young embryos may be formed by two different cell division processes, but normally only one mature embryo develops. Once this embryo begins development within the female gametophyte, the embryo enlarges rapidly and specialized cells form. By fall, mature seeds are formed.

The seed coat begins to differentiate from the integument before fertilization. At pollination a seed wing starts to develop from the ovuliferous scale opposite the micropyle, and the seed with its wing is completely separated from the scale before the embryo is mature. Cones begin to shed seed in September as soon as the seed is mature. The time from pollination to seed release ranges from 120 to 160 days. Shorter maturity periods are associated with drier, warmer weather. Visually, the cones change from red or purple during development to brown at maturity.

Most seeds are shed in the fall when cones first open. Subsequent cycles in wet and dry weather can result in seed release into the next spring. Although cones may remain on the tree throughout the next year, they contain few viable seeds. Western hemlock cones have 20–30 scales. Those in the centre of the cone typically produce about 40 full-sized seeds with less than half of them filled.

Forecasting cone crops Estimating the potential for pollen and seed cone crops is best done in the fall a year previous to harvest. Production of pollen and seed cones on a single tree is generally related, and it is easier to estimate crops based on the more easily recognized pollen cone buds.

The presence or absence of clusters of pollen cone buds at the base of short shoots or from branches collected from the middle portion of the crown gives the most reliable results. Samples must be collected from several trees in an area because individual trees appear to have their own cycle of cone production. Sampling gives an accurate account of the absence of cones, but, because of cone abortion, poor pollination, insects, and disease, the presence of cones does not necessarily mean a good seed production.

This appendix presents the technical details for pollen preconditioning and the three laboratory methods of determining pollen viability. We also provide technical details for controlled pollination, which we use to verify the assay response for the three laboratory tests.

Pollen preconditioning The response for all three viability assays improves somewhat by hydrating pollen just prior to the assay. However, the relationship (regression coefficient, r) between assay response and field fertility (filled seeds per cone) improves substantially only for conductivity (percent conductivity) when the pollen is hydrated (Figures 12, 13, and 14). All pollen lots can be hydrated prior to assay, but we recommend that they always be hydrated before doing the conductivity assay.

The technique we use is to evenly spread about 100 mg of pollen (the dehydrated weight of pollen to be used for the specific viability test). The dish is placed in a closed container saturated with water (100% RH at 20° C). We use a 4-L ice cream bucket with a screen suspended over the water. We do not put the petri dish lid on but we do use a suspended lid to prevent water from forming drops on the pail lid and dripping onto the exposed pollen. For western hemlock, we expose the pollen to these conditions for 16 hours (overnight). It is not necessary to reweigh the hydrated pollen if the weight of the dehydrated pollen is recorded before hydration, and all the hydrated pollen is used in the assay. Rehydrated pollen will nearly double in weight and must be used immediately for the test (i.e., rehydrated pollen cannot be stored for future use).

Conductivity Conductivity analysis to estimate fertility potential of western hemlock pollen is recommended for operational use. The procedure involves measurements of cold and hot conductivity and a simple calculation of the ratio of cold to hot (expressed as percentage). It is a relatively simple process that can be completed in less than 2 hours.

Cold conductivity

1 Hydration: hydrate pollen at 100% relative humidity at 25° C for 16 hours. If tap water is used, let it sit overnight to allow the chlorine to evaporate.

2 Pollen weight: weigh approximately 100 mg of dehydrated pollen, hydrate at 100% for 16 hours, and leach at 25° C for 1 hour. The actual weight of dehydrated pollen can range from 20 to 200 mg. Since pollen weight is affected by moisture content (remember that the weight increases with hydration), all weights should be expressed on a dry weight basis. To calculate the dry weight of pollen, first determine the pollen's moisture content $[(Wt_w - Wt_d)/(Wt_w)] \times 100\%$. The dry weight is then calculated as:

$$Wt_d = Wt_w - (o.MC)(Wt_w)$$

where Wt_d = oven weight of dry pollen
Wt_w = weight of dehydrated pollen (before hydration)

Before hydration begins, weigh the sample to be treated and calculate the dry weight of pollen. Hydrate the pollen for the specified period and use the entire hydrated sample to determine cold conductivity.

3 Leaching volume: 30 mL de-ionized water yields values that are easily determined by commercial conductivity meters. Since variation within lots can be considerable, it is recommended that two replicates of each sample be assayed.

4 Containers: use a plastic container with a tight-fitting, leakproof seal. Do not use a metal container. A 50-mL centrifuge tube with a conical bottom that allows pollen to settle before measurement is recommended.

5 Leaching: transfer the weighed and hydrated pollen directly into the leaching tube. Pipette 30 mL of de-ionized water into the tube. Accuracy in both weighing and pipetting will reduce errors and improve assay results. Replace the cap and ensure that it is leakproof.

To ensure effective leaching, the tubes must be vigorously shaken. Maintain leaching temperature between 22° and 27° C. After 1 hour, remove the tubes from the shaker and allow the pollen to settle for 5 minutes.

Conductivity values will vary with leaching time. Therefore, keep the time between leaching, settling, and measuring constant. If a number of samples are batch tested, then start the measurements on the sample that was pipetted first and proceed in order.

6 Measurement: leachate conductivity can be determined with any commercial meter with a range of 10^{-6} (micro) to 10^{-3} (milli) Siemens (some meters may still use the unit mho but these are not SI units). There are several types of conductivity cells (immersion, flow through, or pipette type), and probes have various ranges of sensitivity (cell constants).

Cell constant refers to the relationship between the cross-sectional area of two parallel plates (electrodes) and the distance between them. Since conductivity results will vary with the cell constant, it is important to first calibrate your system with a standard solution before proceeding.

A 0.01 normal solution of KCl is the standard reference solution for electrical conductivity and has a value of 0.001412 Siemens/cm (S/cm) at 25° C. If your conductivity meter and cell do not produce a value of 1.412 milliSiemens/cm (mS/cm) for the standard KCl solution, then the cell constant is not 1.00. To standardize values, calculate the cell constant ratio K_r:

K_r = (EC Std)/(1.412)

EC Std = electrical conductance (EC) at 25° C for a 0.01 normal KCl solution using your cell

1.412 = EC (mS/cm) value for 0.01 normal KCl where $K_r = 1.00$.

The temperature of the solution will affect the value obtained. The electrical conductance of an aqueous salt solution will increase by about 2% per Celsius degree. Ensure that all solutions are at 25° C. After shaking, place the tubes in a 25° C water bath to allow the pollen to settle and the solution to equilibrate. The settling time (5 minutes) should be consistent between runs.

7 Recording: comparison between lots from different orchards and species is possible by standardizing the results as follows:

$\%\ MC$ = dehydrated pollen moisture content
Wt_d = oven dry pollen weight (gram dry weight [gdw])
Vol = leaching volume (mL)
K_r = cell constant ratio
EC = electrical conductance (mS/cm)
EC_s = standardized EC
T_1 = leaching temperature
T_2 = solution temperature

Thus:

$EC_s = (K_r \times EC)/(Wt_d)$ mS/cm/gdw

Hot conductivity

1 The total leachate can be estimated by heating the pollen for 1 hour in a boiling water bath (an oven at 90–95° C will also work), cooling the pollen to 25° C, and determining the electrical conductivity value. If the solution used to determine the cold leachate does not change volume (i.e., no water is lost because of evaporation), then the same sample can be used to determine the hot leachate value.

2 The results are expressed as cold ($EC_c = COND_c$), hot ($EC_h = COND_h$), and the ratio of cold to hot ($EC_c/EC_h \times 100 = \%COND$).

Germination We have tested several germination media for western hemlock. The essential ingredients for germinating pollen tubes are sucrose, inorganic nutrients (notably calcium and boron), and an osmoticum (to prevent rapid imbibition of water in the germination medium). The addition of sugar to the medium may promote further osmotic regulation and/or provide a source of carbon. Regardless, for western hemlock the addition of sugar to the culture medium improves its germination response.

The medium that provides the best germination response for western hemlock pollen is a 0.25% agar containing a 10% Brewbaker and Kwack's solution (boric acid, calcium nitrate, magnesium sulphate, and potassium nitrate) and 5% sucrose (see Figure 11).

1 Brewbaker's solution compounds	mg/100 mL
Boric acid (H_3BO_3)	100
Calcium nitrate ($Ca(NO_3)2*H_2O$)	300
Magnesium sulphate ($MgSO_4*7H_2O$)	200
Potassium nitrate (KNO_3)	100

Add de-ionized water to make 100 mL of solution.

2 Working solution
The working solution is a 10% dilution of the stock solution. Thus, 10 mL of the working solution are diluted to 100 mL with a solution of 5% sucrose.

3 Agar medium
There are several types and purity of agar. We use a microbiological source, Bacto-Agar product supplied by Difco. The medium is made by weighing 0.25 g of the agar and placing it in 100 mL of 10% Brewbaker's and 5% sucrose (let the water sit overnight if you do not have access to purified water). The solution is then heated to boiling to dissolve the agar and set the gel.

4 Assay Method
About 3 mL of the warm agar solution is plated into a 35 mm diameter petri dish and allowed to cool. Approximately 10 mg of pollen is sprinkled evenly over the agar. The lid is replaced and the petri dish placed in a larger (95 mm) petri dish lined with water-saturated filter paper. The lid of the larger petri dish is replaced, ensuring that the fit is tight. The pollen is incubated for 48 hours at 25°C (on the work bench is also acceptable, but avoid drafts). It is not necessary to control either light intensity or photoperiod.

5 Scoring germination
After 48 hours, pollen germination is scored using a 40x power microscope for a quick view of the germination results, and 100x power for scoring the classes of germinating pollen grains.

Germination of western hemlock pollen is classified into three categories:

Class 1 elongating grain is >2x original diameter
Class 2 elongating grain is <2x original diameter
Class 3 grain is hydrated (original diameter) but not elongating.

The total number of pollen grains scored in each of Class 1 and 2 determines the overall percent germination. Since we are not certain that Class 1 grains are actually more potentially fertile that Class 2 grains, we combine the two for the total percent germination. However, by separating the two for counting purposes, we do get an estimate of the more vigorous lots determined under the specified incubation conditions. Knowing the percentage of high vigour grains with any particular pollen lot may have implications for pollination technique and fertilization potential when several pollen lots are used within a pollen poly-mix.

Respiration Respiration is a measure of oxygen uptake and provides an overall estimate of the metabolic status of pollen. Strictly speaking, the term respiration may not be appropriate, since oxygen uptake can occur in nonrespiratory cellular functions (oxidation). If we can assume that the nonrespiratory oxygen uptake is minor in comparison to respiratory uptake, then respiration is a more useful term and is a direct estimate of the rate of oxygen uptake and mitochrondrial activity.

1 Equipment

Measurement of oxygen uptake in an aqueous solution uses a YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio 45387, USA) model 5300 (dual channel) oxygen monitor, a model 5301 standard bath assembly, two model 5331 oxygen probes (Clark-type polographic electrodes), a constant-temperature, circulatory water bath, and a dual-channel chart recorder.

2 Calibration

The heater/circulatory pump is turned on and set at 30° C. The oxygen monitor is also allowed to warm up for about 30 minutes before calibration or measurements are attempted. The oxygen probes are covered with a gas-permeable membrane to protect the electrode tip. These should be changed regularly, especially if they are scratched or do not meet specifications in the probe test (see YSI manual).

When changing membranes, it is important to ensure that there are no trapped air bubbles between the membrane and probe tip and no wrinkles or holes in the membrane. Change the membrane after 20 or 30 measurements. Under heavy use, this is every 2 days. When the probe is not being used, store the tip in de-ionized water. Refer to probe handling instructions if extended storage is required.

The Model 5300 monitor has a self-zeroing feature but the chart recorder must be zeroed before the probe is activated. The 100% output value is set by immersing the probe into 3 mL of de-ionized water equilibrated at 30° C, ensuring that all the air bubbles have been removed and the stirrer is on. The function switch on the monitor is set to "AIR" and, after unlocking, the calibration channel control knob ("CAL") is rotated to 100% on the display. Relock the "CAL" control knob. Set the recorder to 100%. The monitor output at 100% is 1.000 volt. The stability of the system is indicated by the recorder trace. The trace should be noise-free and not drift more than 0.5% over a 15-minute period.

3 Measurement

About 100 mg of dry pollen is weighed. Record the actual weight of pollen used and its moisture content. Add 3 mL of de-ionized water to the sample chamber and allow the water to reach bath temperature by stirring for 3 minutes. The pollen sample is then added to the water and allowed to reach bath temperature for about 2–3 minutes. Stirring is stopped and the probe is inserted. Make certain that all air is removed (it may take a few stops and starts with the stirring magnet to exclude all air bubbles). The monitor is then switched to "AIR" and the chart recorder turned on (a chart speed of 1 cm/min is suitable). Uptake is allowed to occur over a minimum of 5 minutes. If the pollen lot is particularly active the curve on the chart may reach 0 uptake in less time, in which case a slower chart speed could be used or time as a factor in calculating respiration values changed. The rate of oxygen consumption is calculated from the slope of the trace and read as a change in percent O_2 consumption over time. To complete this, a best-fit straight line is drawn along the trace. A point on the line is selected and the percent O_2 recorded. The percent O_2 for 5 minutes later is also recorded. The difference between the two recordings is the actual percentage of O_2 uptake by the pollen from the aqueous solution.

The respiration rate (i.e., oxygen consumption) is calculated based on the solubility of oxygen in water at 30° C at 1 atmosphere pressure at sea level. Changes in oxygen solubility due to changes in atmospheric pressure are assumed to be small and therefore are ignored. Thus, the solubility of oxygen in water at 30° C is 5.48 µL O_2/mL. The overall respiration rate is then calculated as follows:

$$R_r = (\%O_2 \text{ change})(5.48\ \mu L\ O_2/mL)(3\ mL)/(5\ min)/Wt_d$$

where: R_r = respiration rate (µL O_2/min/gdw)
Wt_d = dry weight of pollen (gram dry weight)

Field testing (controlled cross-pollination) Viability assays are best completed in the spring, before the pollination season, when field testing of pollen lots can be completed. For single lot testing, use the controlled cross-pollination technique.

1 For each lot, use two pollination bags (make sure that all pollen cone buds have been removed) containing three or four seed cone buds on each of four clones (more if time allows) before bud burst. If it is not possible to replicate all pollen lots on the same ramet/individual (clone/family), then use single ramets (individuals) as a replicate. Because male–female interactions are important, do not apply some lots to one clone/family and other lots to a different clone/family. Make certain that all lots are applied to the same clone/family and repeat this on a minimum of four separate ramets/individuals.

2 Pollinate the seed cone buds at their most receptive stage (stage 4, see Plate 5A) with about 0.2–0.5 mL of pollen per bag. If a compressed air pollinator is not available, then use a syringe device and apply the pollen directly to the receptive seed cone buds. We always add a small cube (1 cc) of No-Pest Strip containing 18.6% dichlorvos to each bag to protect against insect damage. The white paper pollination bags can be left on the tree until the cones mature. However, in windy areas bags can tear or damage to the branches can occur so lighter, mesh insect bags may be used as an alternative.

3 For each pollination, a minimum of two cones are sampled and the seed is hand extracted for each cone separately (two replicates per pollination). The number of **potential seed per cone** (PSPC) is determined by counting the number of seeds with fully developed seed coats. The number of seeds with fully developed embryos is determined by either squashing the seed or by X-ray analysis. Results are expressed as filled seed per cone (FSPC).

4 X-ray analysis is the best way to determine the presence or absence of an embryo. We use Kodax Industrex 620 paper and a Hewlett-Packard Faxitron series (Model 43855A) X-ray machine operating at 15 Kv for 2 minutes. Seed yields are expressed as either filled seed per cone (FSPC) or percent filled seed per cone (%FSPC) calculated as the ratio of filled to potential seed per cone (FSPC/PSPC*100). Regression analysis between the viability response and filled seed per cone or percent filled seed per cone determines the strength of the correlation between the two variables.

adaptation a general measure of the ability of an organism to change and cope with the physical and biotic elements of a particular environment, enabling it to reach its potential for growth and reproduction. For orchards, adaptation refers to the degree to which seedlings derived from orchard seed will cope with the environment of the target plantation zone.

adjacency the mature stand of timber adjacent to the cutblock being planted with improved seed.

alleles alternative forms of a gene, but with differing DNA sequence, that controls the same character.

anthesis the stage of full expansion of the flower structures, or—in the case of conifers—strobili. Bud burst is the anthesis of the seed cone (female strobili), and pollen shedding is the anthesis of the pollen cone (male strobili).

archegonium the flask-shaped female sex organ that contains the egg cell. In most conifers, there are more than one archegonium per ovule.

breeding orchard an orchard where (usually) controlled pollination is used to produce progeny for testing. Many more parents are usually maintained in a breeding-orchard than in a seed-orchard.

breeding population the base population of trees in a breeding program that contains the parent material with sufficient genetic variation for long term improvement. For seed orchards, production population is a more specific term and refers to all parent trees within the seed orchard.

breeding value the expected increase of a clone above the mean of the base population for a selected trait. It can be measured in trait units or percentage gain.

bulking (sometimes called vegetative multiplication) clonal reproduction of embryos or seedlings by rooted cuttings, tissue culture, grafting, or somatic embryogenesis.

chromosome rod-like bodies within the cell nucellus that carry the units of inheritance (genes). The number of chromosome sets varies with species and tissue; for western hemlock there are 12 sets for sexual cells and 24 for vegetative cells.

clonal deployment the use of propagules derived from clones for reforestation.

clone a group of plants derived from a single individual (ortet) by asexual reproduction such as rooting, grafting, or tissue culture. All members (ramets) of a clone are genetically identical and can reproduce by mitotic division.

conductivity within the context of pollen viability testing, this is a technique that relates viability with the conductivity of an aqueous solution of pollen leachate.

cone induction bringing about seed cone development by manipulating the environment, cultural practices, or specific plant hormones (e.g., gibberellins).

contamination	the incorporation of pollen external to orchard sources and generally considered to be deleterious to genetic worth either as poor growth performance and/or introducing poorly adapted genes into a production population.
controlled-pedigree orchard	an orchard in which controlled pollination is used for the purpose of producing elite full-sibling crosses.
controlled pollination (CP)	the transfer of pollen from a known source to known seed cone parents while excluding all other pollen sources.
crown management	controlling the size and shape of a tree's crown while maintaining or increasing seed cone and pollen cone development.
cytology	study of cells.
diploid	vegetative cells that have twice the number of chromosomes as the sex cells (haploid) cells.
embryogenesis	a laboratory technique utilizing specific cultures to produce many embryos from a single seed. These are developed into seedlings that are genetically identical to the original parent seed. Seedlings derived by this process are called emblings.
family	seedling offspring from a particular tree. Half-siblings have one parent in common among family members and full-siblings have both parents in common.
fertility potential	the potential for a particular pollen lot to fertilize an egg cell.
fertilization	the union of the male gamete (sperm) with the female gamete (egg) to form a zygote from which a new plant will form.
filled seed per cone (FSPC)	the number of seeds per cone with a fully developed embryo.
gamete	a mature sex cell, either male (sperm) or female (egg). For conifers, there are two sperm cells (derived from the body cell) within each pollen grain.
gene	a hereditary unit that consists of a specific portion (length) of one of the organism's chromosomes.
genetic diversity	tree breeders use this term to refer to both the variability of traits within a species and the variability of genetic material for these traits.
genetic efficiency	the extent to which orchard seed genetic quality and genetic variability reflects the parental composition of the orchard.
genetic gain	the average (heritable) change from one generation to the next that results from selection.
genetic variability	a general term that reflects both the number and the genetic heterogeneity of the contributing parents to orchard seed.

genetic worth the average breeding value of all seed from contributing parents weighted for the proportion of their contribution.

genotype an individual characterized by a specific genetic constitution. The manifestation of a genotype interacting with the environment produces a phenotype.

germination within the context of pollen viability testing, this is a technique that relates viability with the growth of pollen grains under controlled conditions.

gibberellins a class of plant hormones, which number over 100, that occur in most plants including conifers. The mixture of gibberellins A_4 and A_7 (GA4/7) is the active component for inducing flowering in western hemlock as well as most other Pinaceae species.

haploid cells that have undergone reduction division (meiosis) and carry one-half the number of chromosomes (gametes) of the vegetative (diploid) cells.

hedge-orchard an orchard of hedged plants that produces cuttings or other tissue for vegetative propagation. Hedge-orchards have also been used in New Zealand for controlled crossing seed production. In these orchards ramets are placed in clonal rows and tightly spaced (500 sph), crowns are pruned for height control, and induction begins the year after grafting.

hedging the repeated clipping of an ortet and/or some of its ramets to produce a low hedged plant. The purposes of this procedure are to slow or halt maturation of the plant, and to control its size and shape for efficient operations.

heterogeneity a qualitative term that describes the extent of variability for a number of traits within a species. For example, genetic heterogeneity describes differences between individuals as a result of the combined action of all genes.

homozygosity presence of identical alleles (either dominant or recessive). Homozygous individuals breed true for the trait in question when mated with the same genotype.

imbibition the activity of water uptake in which water moves from an area of high potential (e.g., pure water for germination) into an area of low water potential (dry pollen).

inbreeding intercrossing of related clones (including self-pollination) of a sexually reproducing population. In general, inbreeding decreases genetic variability and increases homozygosity.

incremental value the value of tree improvement that is beyond past and current investment.

integument the tip of the ovule structure surrounding and derived from the nucellus. During early (stage 2) seed cone elongation the integument forms a wide funnel-shaped structure (the micropyle) surrounding the nucellus.

meadow orchard a variant of the traditional orchard design where ramets are planted at considerably higher density (5000 stems per hectare), cone induction begins the year after establishment, and trees are pruned as required to keep tree height under 2 metres.

meiosis nuclear divisions leading to the formation of gametes (either egg or sperm cells). The first division results in reducing the number of chromosomes in half (haploid) and the second division results in the formation of four haploid daughter nuclei.

meristem an undifferentiated plant tissue, often with rapidly dividing cells, from which new tissues or organs arise.

micro-orchard an orchard in which the ramets are established within clonal rows, crowns are maintained under 3 metres, and density of ramets is 400–500 stems per hectare.

micropyle a wide, funnel-shaped structure arising from the integument and surrounding the nucellus through which the geminating pollen tube must pass to penetrate the nucellus to reach the egg cell.

microsporangia two microsporangia are borne on each microsporophyll of the pollen cone. They contain the tissue that forms the pollen mother cells, which undergo meiosis and form the single-celled microspore that eventually develops into the 5-celled pollen grain.

microspore the male pollen grain, which is mature at shedding and consists of 5 cells; two prothallial cells, a stalk cell, a body cell, and a tube cell.

microsporophyll the bract-like structures of the pollen cone upon which the pollen sacs (microsporangia) are borne.

mitosis mitosis is the division of a nucleus into two daughter nuclei by a process of duplication and separation of chromosomes sets. Cell division and cell enlargement then occur, resulting in vegetative growth.

mother cell the tissue cells from which sex cells develop.

nucellus at the base of the micropylar canal, the nucellus is the first tissue mass that a pollen tube must penetrate to reach the egg cells.

open pollination pollination where exposed and receptive seed cone buds are pollinated by any pollen source. In wild stands, wind is the principal vector, whereas in seed orchards, wind is augmented by artificial methods (supplemental technique).

ortet the original parent tree in wild stands from which clonal copies (ramets) are produced using either grafting, rooting, or tissue culture techniques.

osmotic potential the solute potential of the medium and cell, which affects the direction and magnitude of water movement in and out of the pollen grain.

osmoticum a solute of polyethylene glycol added to a germination medium to adjust the osmotic potential of the medium to that of the pollen grain.

out-crossing breeding between parents that are not related (selfing or inbreeding).

outcross pollen pollen that is available for fertilization (either airborne or applied supplementally) that is unrelated to the seed cone parent tree.

ovuliferous scales	the scales of the seed cone buds that carry the ovules and then the seeds. They are analogous to the microsporangia of the pollen cone bud.
percent filled seed per cone	seed efficiency defined as the ratio of filled seed per cone divided by the potential seed per cone.
percent gain	the percent improvement of a selected trait for an orchard population in comparison to some unimproved population.
phenology	study of the relations between climate and periodic biological phenomena.
phenotype	the observable characteristics of an organism produced by the interaction of its genotype with the environment.
phytotoxic	the damage resulting from application of chemicals, such as gibberellins and surfactants, to plant tissue (foliage).
pollen cloud density	a relative measure of the number of airborne pollen grains. The actual value is dependent on the technique used, in which case the term could be relative pollen cloud density.
pollen load	a relative measure that indicates pollen cloud density. One hundred percent pollen load is the value beyond which increases in density do not produce corresponding increases in seed yields.
pollen management	the use of various pollen handling techniques to aid or control pollination. See also controlled pollination and supplemental (mass) pollination.
pollen mix	the application of mixed clonal sources of pollen parents either under controlled crossing or open pollinated conditions.
pollen tube	an elongated outgrowth of the germinating pollen grain that penetrates the nucleus and ovule and through which the sperm is transferred to the egg cell.
pollen vigour	a quantitative measure of the actual response of a pollen lot to laboratory assays (e.g., high germination or respiration rates refer to a high vigour pollen lot).
pollination efficiency	the degree to which pollination or supplemental pollination effectively delivers pollen to the seed cone, resulting in seed formation. For most conifer species, pollination efficiency of 100% would be considered to be three to five pollen grains reaching the micropyle but for western hemlock this may only mean pollen deposited on the bracts and scales.
potential seed per cone (PSPC)	the number of seed per cone with fully developed seed coats. For western hemlock, this number is an estimate of the actual number of viable ovules at pollination.
progeny	the offspring of sexual reproduction.
progeny trial	a trial in which the performance of pedigreed progeny from different parents are compared, usually over several progeny sites. Results from progeny trials are used to backward-select among the parents or to forward-select among the offspring.

propagule a young plant produced by any one of a number of propagation techniques.

ramet a genetically copied individual from a parent tree clone.

receptivity the stage of phenological development of the seed cone that permits the entry and capture of pollen on exposed bracts, scales, and micropyle.

respiration within the context of pollen viability testing, this is a technique that relates viability to oxygen uptake in an aqueous solution.

roguing the systematic removal of undesirable clones or individuals from the orchard breeding population because of low breeding value or poor flower production.

scales ovuliferous scales.

seed orchard a plantation of clones or seedlings from selected trees, cultivated for early and consistent flowering and managed to produce seed crops with a desirable genetic quality and variability.

seed set the culmination of all steps of sexual reproduction from pollination to seed maturation. For purposes of evaluating field pollination trials, we use filled seed per cone as the sampling unit for seed set.

seed transfer guidelines the restrictions put on the deployment of any seed lot to specific seed zones and elevation bands to ensure that the seedlings are well adapted to the plantation site.

selection trait a specified trait (e.g., growth) that is the criterion used to compare performance of offspring of selected parents (clones).

self-fertilization fertilization by self-pollination.

self-pollination pollination of female strobili of a tree with that tree's own pollen.

strobilus the reproductive structures of conifers containing the micro-gametophytes (pollen cones) and the mega-gametophytes (seed cones).

supplemental pollination (SP) (also called supplemental mass pollination [SMP]) the broadcast spraying of large amounts of pollen (several litres) used to both increase orchard yields and/or genetic worth.

surfactant an agent (e.g., Aromox) added to aqueous solutions to help "wet" foliage when applying foliar sprays (for cone induction).

total seed per cone the total number of fully developed seed coats (from fully developed ovules) and rudimentary seed (from immature ovules).

vegetative multiplication (bulking) when seeds of desired families or populations are in short supply, embryos or seedlings are clonally reproduced by rooted cuttings, tissue culture, grafting, or somatic embryogenesis.

viability the ability of a pollen grain or pollen lot to set seed as determined by its assay performance in laboratory tests (viability assays). For western hemlock, three assays are used: respiration, conductivity, and germination. Correlation analyses are used to determine the strength of the relationship between laboratory assay response and field fertility (seed set).

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